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Dissolved Phosphorus Dynamics in Headwater Streams[1]

Yves T. Prairie[2] and Jacob Kalff

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Prairie, Y. T., and J. Kalff. 1988. Dissolved phosphorus dynamics in headwater streams. Can. J. Fish. Aquat. Sci. 45: 200–209.

A comparative study of seven headwater streams was undertaken to evaluate the relative importance of land use, water discharge, water temperature, solar irradiance, and stream sediment resuspension on levels of dissolved phosphorus (DP). In six of the streams, [DP] increased from early spring to late summer. Nutrient-rich streams are temporally more variable than nutrient-poor ones. Average DP concentrations were not related to the proportion of forest cover but showed a correspondence with underlying geology. Despite the restricted geographical area, relationships between [DP] and water discharge varied in magnitude and in direction among the streams. Simple hydrological models treating the catchments as two-reservoir systems accounted for the different behaviors and performed satisfactorily when the effect of temperature was considered. The residual variance showed no seasonal trends but was significantly correlated with daily solar irradiance for one of the seven streams. Finally, the hypothesis that resuspension of stream sediments affects soluble phosphorus concentration was experimentally rejected.

On a effectué une étude comparative de sept cours d'eaux d'amont afin d'évaluer l'importance relative de l'utilisation des terres, du débit, de la température de l'eau, du rayonnement solaire et de la resuspension des sédiments par rapport au niveau de phosphore dissous (PD). Dans six des cours d'eaux, [PD] a augmenté du début du printemps jusqu'à la fin de l'été. Les cours d'eaux riches en bioéléments sont temporellement plus variables que ceux pauvres en bioéléments. Les [PD] moyennes n'étaient pas liées au pourcentage de couvert forestier quoiqu'elles montraient un rapport avec la géologie sous-jacente. Malgré la superficie géographique restreinte, les relations entre [PD] et le débit ont varié en importance et en direction parmi les cours d'eaux. De simples modèles hydrologiques où les aires d'alimentation sont considérées comme des systèmes à deux réservoirs expliquent les différents comportements et ont donné de bons résultats quand l'incidence de la température était incluse. La variance résiduelle n'a pas révélé de tendances saisonnières quoiqu'elle était en corrélation significative avec le rayonnement solaire quotidien dans le cas d'un des sept cours d'eaux. En dernier lieu, on a rejeté l'hypothèse selon laquelle la resuspension des sédiments influe sur la concentration de phosphore soluble.

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Atkins (1925) noted that uncontaminated streams contain very low but quite variable concentrations of phosphorus. This observation, however, did not spark much interest among limnologists until phosphorus was convincingly identified as a major factor in the eutrophication of streams and lakes (Vollenweider 1968). Models predicting phosphorus concentration in lakes (e.g. Vollenweider 1969; Dillon and Rigler 1974) link land and lakes in that they require estimates of phosphorus transport in inflowing streams. Such estimates are, in turn, usually derived from empirical models (e.g. Dillon and Kirchner 1975; Kirchner 1975; Prairie and Kalff 1986) that often exhibit large prediction errors. Reduction of this uncertainty will, in all likelihood, depend on a fuller integration of the factors influencing phosphorus levels in running waters, something the present paper examines.

Processes involved in the dynamics of phosphorus in headwater streams include interactions with the biota (Meyer 1979, 1980; Klotz 1985), the stream sediments (Taylor and Kunishi 1971; McColl 1974; Meyer 1979; Logan 1980), the hydrology of the catchment (Bouldin et al. 1975; Rigler 1979; Munn and Prepas 1986), and the soil geochemistry (Wood et al. 1984). The importance of watershed disturbance on solutes in running waters has also been documented (Likens et al. 1970; Hobbie and Likens 1973; Bormann et al. 1974). However, these intensive studies usually involved only one or two streams, so the relative importance and the generality of individual findings are largely unknown.

Here we report the results of a comparative study of seven headwater streams, draining catchments of different characteristics. We attempt to explain and model the temporal changes in dissolved phosphorus (DP) concentration in these streams as

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a means of evaluating the relative importance of land use, the hydrology, climatic factors, as well as of the stream sediments in controlling phosphorus levels in streamwater. More specifically, we test (1) whether seasonal changes in [DP] are consistent among streams, (2) whether land use is a good predictor of average soluble phosphorus levels, (3) whether [DP] – water discharge (Q) relationships are consistent among streams, (4) the influence of temperature on [DP], and (5) whether stream sediment resuspension can account for changes in [DP] through sorption/desorption reactions. In addition, we test a simple hydrological model to explain variation in DP – Q relationships. Only DP is examined here, with the dynamics of particulate phosphorus (PP) the object of a companion paper (Prairie and Kalff 1988). This separation of phosphorus into dissolved and particulate fractions is justified on the basis of their different behavior with respect to hydrology (e.g. Meyer and Likens 1979) and biological availability (Sonzogni et al. 1982).

Study Sites

The study sites (Fig. 1) are located within a 15-km radius of Lake Memphremagog Field Station (45°06′N, 76°17′W) in the Eastern Township region of Quebec. The area receives an average of 110 cm in precipitation annually, of which about 25% falls as snow. The distribution of precipitation is essentially uniform throughout the year, and the fraction of water loss from transpiration and evaporation is in the order of 40%. The streams are all headwaters of first- or second-order draining catchments varying in size, land use, vegetation cover, and underlying geology (Table 1). In addition, it should be noted that C2 is a small stream which intermittently dries up in midsummer. In the dry summer of 1983, no flow was observed in C2 from mid-June to late July. C3 is slightly larger but also occasionally runs dry. C7 is the most complex catchment in that the stream is fed in part by mountain springs and by draining field areas. The catchment also supports a small rural community. None of the streams receives phosphorus from point sources.

Methods

The sampling period extended over two field seasons, from May 1 to August 30 in 1983 and from March 20 to August 30 in 1984. The streams were further visited twice during the fall of 1984. The data collection program basically consisted of two different sampling schemes, depending on the occurrence of rain. First, each stream was sampled on a regular basis approximately every 3–4 d. During the snowmelt period of 1984 the streams were sampled once a day because of the rapidly changing flow conditions. In addition to this background data collection, the streams were intensively sampled during a total of 22 rain events, varying in magnitude from ephemeral showers to heavy rain storms. Upon the onset of a rain event, a particular stream was chosen for intensive monitoring, and thus, an average of about three rain events were studied for each stream. The sampling frequency during these rain events also varied depending on the rate of change of water discharge, with more frequent collection at times of rapidly fluctuating discharge. The median time between sampling was 70 min.

Unless stated otherwise, the data collected during the rain events were used to calculate volume-weighted daily estimates of conductivity and phosphorus concentration. The weighted estimates were determined by linear interpolation of water discharge and of phosphorus and conductivity fluxes between sampling sessions. In this manner, the measurements gathered during rain events can be compared with the background data for which the single sampling was assumed to represent the prevailing conditions of the day.

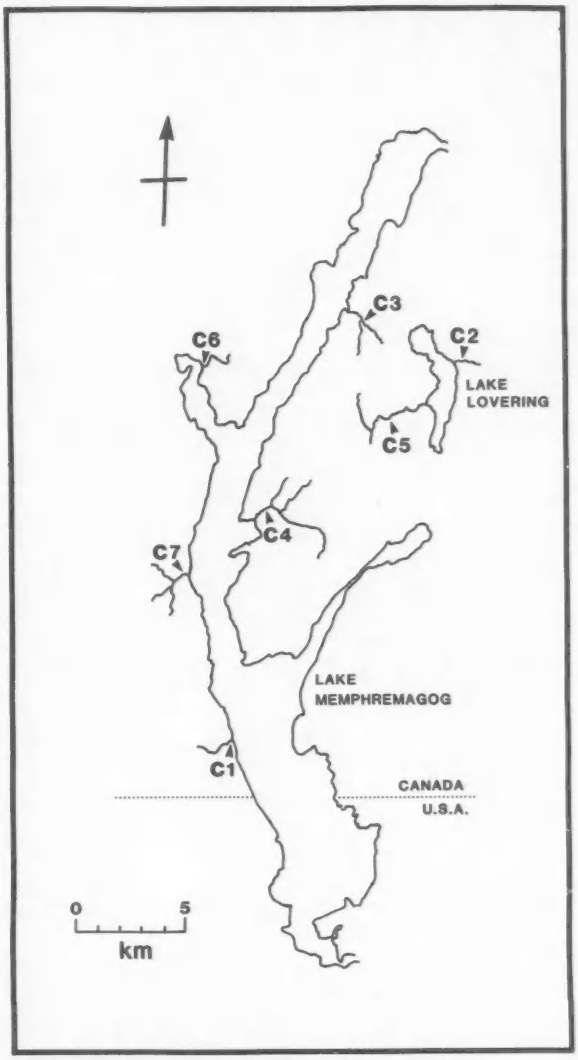

FIG. 1. Locations of the streams and sampling sites.

Water discharge (Q, litres per second) was determined at every sampling using the salt injection technique (Church and Kellerhals 1970). The method consists in rapidly injecting a volume of a saline solution of known conductivity and recording the passage of the "salt" wave at a downstream station until the conductivity returns to background levels. Multiple replication at low and high discharge rates and in different streams gave coefficients of variation of less than 10%.

Water samples were collected in triplicate 50-mL tubes and in a 1-L dark Nalgene bottle at middepth in the channel for total phosphorus (TP) and total DP, respectively. Upon return to the laboratory, the 1-L bottle was filtered through washed 0.45-μm millipore filters into three 50-mL samples for DP analysis.

TABLE 1. General characteristics of the catchments studied.

Site	Area (km^2)	Median Q ($L \cdot s^{-1}$)	Land use	Geology
C1	2.0	11	Deciduous forest	Igneous
C2	1.5	10	Hay field	Sedimentary
C3	2.8	23	1/2 deciduous forest 1/2 ungrazed pasture	Sedimentary
C4	14.5	139	3/4 mixed forest 1/4 abandoned field	Sedimentary
C5	6.3	93	Coniferous forest	Sedimentary
C6	4.1	34	1/2 coniferous forest 1/2 cultivated field	Sedimentary
C7	8.6	109	2/3 mixed forest 1/3 field	Sedimentary/ igneous

Phosphorus concentration was determined according to the method of Strickland and Parsons (1968) following potassium persulfate oxidation and autoclaving (Menzel and Corwin 1965). The detection limit for this method is 1 $\mu g \cdot L^{-1}$ and has an analytical coefficient of variation approximated by $1/5\sqrt{DP} \times 100$. Particulate phosphorus (PP) was calculated as the difference between TP and DP, which is essentially equivalent to fine PP, since coarse particulate material (>1 mm) was never observed in the TP samples.

Conductivity was measured in the field with a portable temperature-compensated Markson conductivity meter.

Sediment Resuspension Experiments

We conducted a series of laboratory experiments to test whether stream sediment resuspension can significantly alter stream water DP concentration. The experiments were carried out by resuspending increasing amounts of stream sediments (0.3, 1.5, 2.0, and 3.0 g fresh weight) into 12 L of stream water under conditions of continuous stirring. The stirring was assumed to adequately simulate the mixing regime of turbulent streamflow in which suspended sediment travels more or less at the same velocity as the water (Ryden et al. 1973). After each sediment addition, aliquots were drawn at fixed time intervals (1, 3, 7, 15, 30, and 60 min for TP and DP analysis.

Nonlinear models were fitted using the NLIN procedure (Marquardt method) of SAS (version 5.08; SAS Institute Inc. 1985). All other analyses were also performed with SAS packages.

Results and Discussion

Temporal Variation in DP Concentration: Mean and Variance

Several notable features emerge from a first examination of the temporal changes in [DP] of the seven streams (Fig. 2). First, most streams (all but C2) exhibit significant positive correlations with time between March and August (Table 2), as found by Taylor and Kunishi (1971). In contrast, Hobbie and Likens (1973) and Meyer and Likens (1979) found no seasonal patterns. This general seasonal tendency is further characterized by a correlation matrix of [DP] among all streams for matching sampling dates. All correlations are significant (Table 2), except again for those involving C2. Thus, changes in [DP] in each of the six streams are, to a large extent, mirrored in the other streams, suggesting the influence of a factor common to the rather disparate streams. The highest correlations are observed among catchments with little (C7) or no (C1 and C5) ecosystem disturbance.

Second, nutrient-rich streams are temporally more variable than nutrient-poor ones (Table 3). This trend is noticeable also within a given stream, with both the mean and the variance

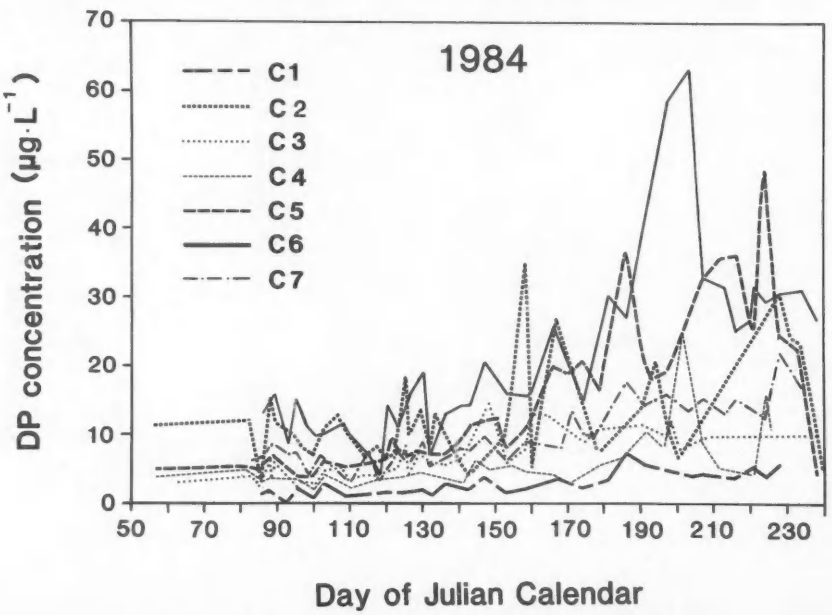

FIG. 2. [DP] time series for the 1984 field season in the seven streams.

TABLE 2. Correlation matrix of observed [DP] in the different streams for matching sampling dates. The first row is the linear correlation coefficient, the second row the associated probability level, and the third row the number of observations.

	Julian day	C1	C2	C3	C4	C5	C6	C7
C1	0.58	1	0.44	0.56	0.54	0.69	0.53	0.71
	<0.001		0.025	0.002	0.002	<0.001	<0.001	0.000
	55		26	28	32	32	48	46
C2	0.19		1	0.24	−0.05	0.29	0.29	0.55
	0.184			0.135	0.802	0.054	0.155	0.006
	51			41	31	45	26	24
C3	0.61			1	0.36	0.54	0.51	0.56
	<0.001				0.043	<0.001	0.006	0.003
	50				32	43	28	26
C4	0.42				1	0.47	0.62	0.59
	0.001					0.001	<0.001	0.001
	58					45	29	31
C5	0.67					1	0.58	0.77
	<0.001						0.001	0.000
	72						31	28
C6	0.63						1	0.72
	<0.001							0.000
	58							51
C7	0.62							1
	0.000							
	60							

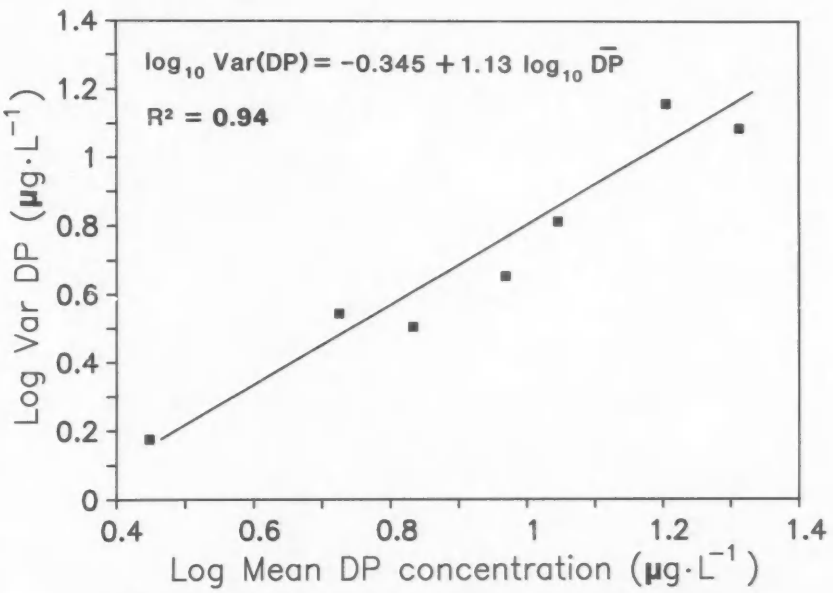

FIG. 3. Scatter plot of the temporal variance versus mean [DP] concentration.

increasing as the summer progresses (Fig. 2). The magnitude of this phenomenon is particularly well shown when the temporal variance is plotted against the average [DP] of each stream (Fig. 3) and is further discussed in the section on hydrology. A regression line passing through the data of Fig. 3 can be useful in the design of future stream phosphorus sampling programs. When we applied to our streams a method analogous to that used by Downing (1979) for benthos, we calculated that the number of samples necessary to obtain an estimate of the mean [DP] with a coefficient of variation of 20%

TABLE 3. Mean [DP] and associated variances (Var[DP]) in the seven streams. N is the pooled number of sampling dates for the two field seasons.

Site	N	[DP] ($\mu g \cdot L^{-1}$)	Var[DP]
C1	55	2.8	1.5
C2	51	11.1	6.5
C3	50	6.8	3.2
C4	58	5.3	3.5
C5	72	16.0	14.4
C6	58	20.5	12.2
C7	60	9.3	4.5

or less is $N = 4.85[\bar{DP}]^{0.27}$ where DP is an estimate obtained from a pilot survey. Consequently, only six sampling visits are required for a DP-poor stream (DP ≈ 2 $\mu g \cdot L^{-1}$) but about 12 for one with 30 μg DP$\cdot L^{-1}$.

The degree of ecosystem disturbamce does not appear to be particularly important in determining the average DP levels, as the two completely forested catchments (C1 and C5) have the lowest and second highest mean [DP], respectively (Table 3). Contrary to Omernik (1976) and Hill (1981), our data failed to show a significant correlation between the proportion of forest cover and the mean [DP]. This is not entirely surprising in view of the vast scatter of existing land use – [DP] relationships (Omernik 1976, 1977). Our findings are also consistent with those in Hubbard Brook (Hobbie and Likens 1973; Bormann et al. 1974) in which changes in soluble phosphorus levels were modest after deforestation.

The geology of the drainage areas is more successful in classifying the streams with respect to their dissolved nutrient status (Tables 1 and 3). Stream C1 is the only one underlain by an igneous formation and also has the lowest soluble phosphorus levels. The geology of C3 and C4 consists largely of limestone and both have very similar mean [DP]. Streams C2, C5, and C6 have the highest phosphorus levels and all three are underlain by slate. Finally, the geological understructure of C7 is a mixture of igneous rocks and slates with an intermediate mean [DP]. DIllon and Kirchner (1975) reported an analogous association with geology in that catchments underlain by igneous bedrocks exported very little phosphorus. While our streams are too few to permit a conclusive generalization, the hypothesis of a widespread correspondence between geology and soluble phosphorus levels should be investigated further, as it may result in a useful empirical rule (Moog 1984).

Influence of Hydrology

The influence of hydrology on the dynamics of DP in headwater streams (in particular DP – Q relationships) is not well understood. Taylor and Kunishi (1971), Hobbie and Likens (1973), McColl et al. (1975), and Meyer and Likens (1979) found no significant relationships between soluble phosphorus and water discharge. Conversely, Rigler (1979), Munn and Prepas (1986), Smith (1976), and McColl et al. (1977) found positive trends (runoff effect) between the variables. Finally, Taylor and Kunishi (1971) studied streams in which DP was reduced with increasing discharge.

Interestingly, all three types of relationship occurred in our streams. Rank correlation coefficients between [DP] and Q were significant ($p < 0.05$) in six of the streams ranging from -0.84 to $+0.43$ but were not significant for stream C4 (Fig. 4). The values used in this analysis are measurements collected throughout the sampling seasons. As a result, the seasonal analysis describes the relatively long-term effect of hydrological regimes on phosphorus dynamics. This type of data treatment would, however, mask much of the hydrological effects occurring at a much shorter time scale, such as during rain storms. We examined this possibility by repeating the same correlation analysis but this time using the samples collected during rain events. Between two and four rain storms were sampled for each stream, yielding an average of 23 rain data points per stream. To assess the validity of this analysis for comparative purposes, we first considered whether the range of water discharge generated by the rain events was roughly equivalent to that observed over the two seasons. In C2 to C6, the proportion of the total range of water discharge which was represented by the rain data set exceeded 84%, but in C1 and C7 this fraction amounted to only 11 and 23%, respectively. Thus, comparisons of the two data sets (routine and intensive sampling) in these two sites are likely to mislead. Indeed, the correlations between DP and Q in the rain data set are identical in direction and statistical significance to those obtained in the seasonal analysis for all but streams C1 and C7. In these two, we attribute the shift from significantly negative trends in the no-rain data to nonsignificance in the rain data to the incommensurate water discharge ranges. It appears therefore that the effects of hydrological regime on [DP] are similar for the short and intermediate terms.

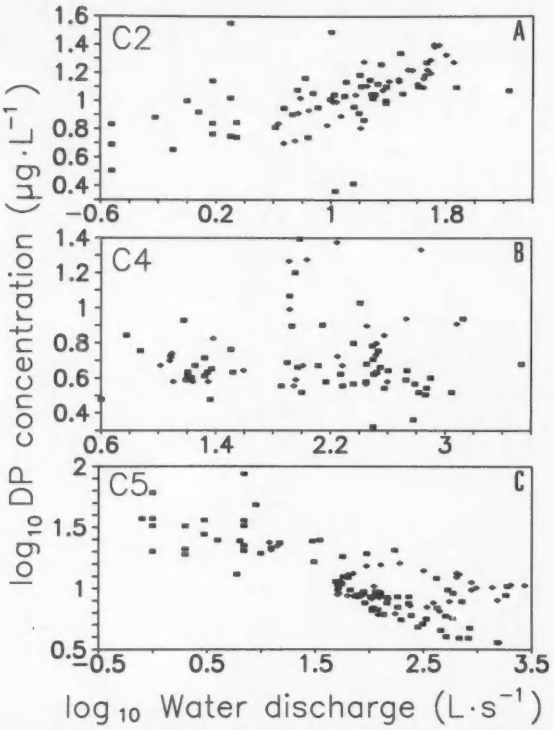

FIG. 4. [DP]–Q relationships for three streams selected so as to emphasize the heterogeneity of the trends. For visual clarity, especially at the lower end of the abcissa, the axes have been transformed to logarithms. Squares are samples collected routinely and diamonds during rain events.

Before interpreting the DP–hydrology relationships any further, we examined some other properties related to the hydrology of the catchments. In all seven streams, conductivity exhibit

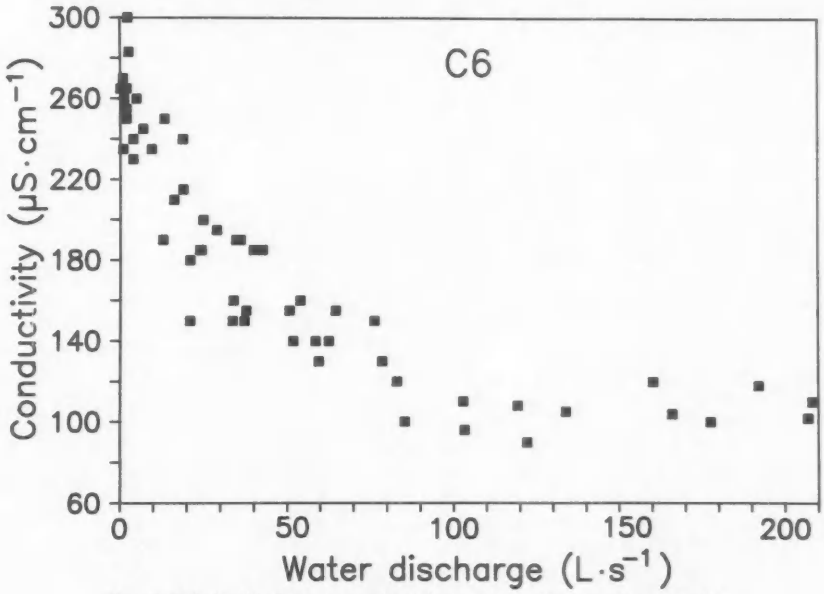

FIG. 5. Relationship between conductivity and water discharge in stream C6.

rapidly decreasing functions with increasing discharge (e.g. C6, Fig. 5). The conductivity data are thus easily modeled by treating the drainage basins as systems of two reservoirs ("near-surface" and "deep-seepage") of different nominal conductivities. Depending on the degree of mixing of the two sources and how it varies with total streamflow, the conductivity of the streamwater is predictable from a simple two-system mixing model of the form

$$(1) \quad C_{stream} = (\alpha C_{deep} + QC_{surf})/(Q + \alpha)$$

where C_{stream} is the observed stream conductivity ($\mu mS \cdot cm^{-1}$) and C_{deep}, C_{surf}, and α are fitted constants estimated via nonlinear regression analysis representing the nominal conductivities of the "deep-seepage" and "near-surface" reservoirs and a mixing parameter, respectively. The value of the mixing parameter α is the discharge at which both reservoirs contribute equally. In this model, the proportion of water contributed by the "near-surface" reservoir increases with total streamflow as $Q/Q + \alpha$ and thus dominates when $Q > \alpha$. Conversely, the proportion contributed by the "deep-seepage" reservoir decreases as $\alpha/Q + \alpha$. This empirical model is conceptually analogous to the more mechanistic "Birkenes" model (Christophersen and Wright 1981; Christophersen et al. 1982) in that streamwater chemistry is the result of the mixing of two "reservoirs" contributing differentially depending on streamflow. In our streams, the estimated conductivity was higher in the "deep-seepage" reservoir than in the "near-surface" waters. The general model (Eq. 1) was fitted to the data of each stream and yielded a satisfactory agreement between observed and predicted values, with correlation coefficients ranging between 0.80 and 0.95.

A simple hypothesis to account for changes in [DP] would be to assume the behavior of DP to be analogous to that of conductivity. However, when tested with the stream DP data, the simple mixing model did not perform as well and as consistently and thus resulted in a goodness of fit which varied greatly between streams (Table 4). This may be expected in view of phosphorus–soil/sediment interactions (Williams et al. 1970; Wood et al. 1984; Ryden et al. 1973; Taylor and Kunishi 1971). That the model fits relatively well in at least some of the catchment (e.g. C5, Table 4 and Fig. 4B) is, however, indicative of the variable nature of the processes associated with changes in [DP] in different streams. The lack of relationship between DP and Q precluded the use of Eq. 1 for stream C4.

TABLE 4. Parameter estimates of the simple mixing model (Eq. 1) applied to DP. r is the correlation coefficient between observed and predicted values, SE_{DP} the standard error of prediction, and N the number of observations. In stream C4, no parameter estimates can be obtained because of the independence of DP and water discharge.

Site	DP_{deep}	DP_{surf}	α	r	SE_{DP}	N
C1	4.4	1.7	6.0	0.43	1.39	53
C2	5.8	14.4	9.2	0.54	3.58	47
C3	9.4	2.2	39.0	0.57	2.69	48
C4	—	—	—	—	—	—
C5	36.2	4.3	25.9	0.75	9.64	71
C6	26.7	2.4	122.6	0.38	11.58	56
C7	13.4	4.1	123.4	0.57	3.74	59

Even in sites where the predictive ability of the simple mixing model is relatively poor, the form of Eq. 1 does account for some of the more qualitative differences among streams. In particular, the opposite direction of the DP–Q relationship in C2 in contrast with C1,C3,C5,C6, and C7 can be explained by postulating a reversal in the relative richness of the "near-surface" and "deep-seepage" reservoirs. The estimates of the [DP] of the two contributing reservoirs (Table 4), obtained through nonlinear estimation, support this contention for C2. A system with richer "near-surface" waters is described in Rigler (1979). Table 4 demonstrates also that the "near-surface" waters of five of the seven streams are consistently DP poor and surprisingly stable among catchments. In contrast, the

phosphorus levels in the "deep-seepage" reservoirs are higher and much more variable. Given the low, relatively stable amounts of phosphorus in the "near-surface" waters and the varying contributing proportions of deep and shallow waters at different flow rates, the simple mixing model accounts for the greater temporal variation observed in richer streams (Fig. 3).

Influence of Temperature

Given that water discharge decreases over the summer and that we found an inverse relationship between DP and Q in most of our streams, the tendency of DP to increase from early spring to late summer (Fig. 2) can be anticipated. This hypothesis, however, cannot alone account for the patterns observed. In C2, where DP and Q are positively correlated, no significant seasonal trend is apparent. In C4, where DP and Q are unrelated, the seasonal trend is maintained. If the changing hydrological regime alone had been responsible for the seasonal patterns, C2 and C4 should have exhibited a negative and a lack of significant trend with time, respectively. These discrepancies are, however, easily accounted for by postulating a process, such as temperature, that increases the [DP] over the summer independently of the hydrological conditions. Under such conditions, the seasonal trends in [DP] should be most pronounced in C1,C3,C5,C6, and C7, less pronounced in C4, and further reduced (or nullified) in C2. The empirical observations are in agreement with the postulated trends (Table 2; Fig. 2).

Other lines of evidence suggest that temperature influences the [DP] of stream water. The laboratory experiments of Barrow (1974) and Barrow and Shaw (1975) show that soil phosphorus sorption reactions are exothermic, with high temperature yielding high solution phosphorus concentrations. When the present data are divided into spring (March and April) and summer (June and July) periods so as to emphasize the extremes in temperature and DP compared for days with similar water discharge rates, the phosphorus concentrations are generally higher in summer than in spring (e.g. Fig. 6).

Combining the result of Barrow and Shaw (1975) and the assumption that the [DP] of the "near-surface" reservoir (of the hydrological model) is a temperature-dependent function yielded the following model:

$$(2)\quad [DP] = (\alpha[DP]_{deep} + Q(\beta_0 + \beta_1 \cdot TEMP))/(Q + \alpha)$$

where TEMP is water temperature (degrees Celsius) and α, β_0, β_1, and $[DP]_{deep}$ are fitted constants. In this model, the influence of temperature on [DP] in the "near-surface" reservoir is assumed to be linear ($\beta_0 + \beta_1 \cdot TEMP$) for the range of water temperature observed in our streams.

The stream DP data were fitted to Eq. 2 and the estimated parameters as well as the correlation coefficients between observed and predicted values are reported in Table 5. Because [DP] and water discharge are independent in C4, the influence of temperature was modeled, for this particular stream, with a simple linear model of the form $DP = \beta_0 + \beta_1 \cdot TEMP$. The inclusion of a temperature term significantly ($p < 0.05$) improved the predictive power of the model in comparison with the simple mixing model (Eq. 1) for five of the streams (Tables 4 and 5), reducing the residual variance by an average of 27%. A similar improvement was noted by McColl et al. (1977) when they included soil temperature at 1 m of depth in a multiple regression model. We have been unable so far to relate the temperature coefficients of Eq. 2 to any particular catchment characteristics we had measured. To avoid possible misinterpretation of our results, we checked for serial correlation among the residuals of each stream-specific model (Table 5). However, because our data were not obtained from a fixed interval sampling, we could not use a standard analytical test (e.g. Durbin–Watson). Instead, we produced for each stream a set of three (1-, 2-, and 3-d lag), albeit incomplete, serial correlation plots. In all cases except stream C6, no significant correlations were found, establishing that serial correlation was not a serious problem in most cases. In C6, however, we detected significant positive correlations between the residuals of consecutive (t –

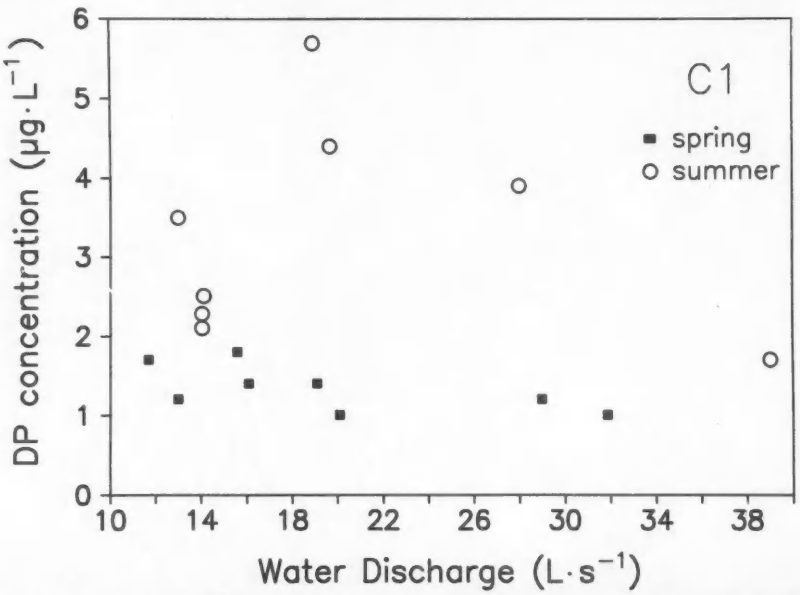

FIG. 6. Comparison of [DP] in stream C1 for samples collected in spring (squares) and summer (circles) for a range of water discharge common to both periods.

TABLE 5. Parameter estimates of temperature-dependent model (Eq 2). r is the correlation coefficient, SE_{DP} the standard error of prediction, and N the number of observations.

Site	$[DP]_{deep}$	β_0	β_1	α	r	SE_{DP}	N
C1	3.8	0.81	0.10	4.7	0.54	1.17	44
C2	6.5	13.18	1.79	70.6	0.69	3.18	41
C3	11.8	2.25	0.15	11.0	0.80	2.01	39
C4	—	3.54	0.07	—	0.36	1.29	46
C5	37.7	2.12	0.10	26.5	0.78	10.13	58
C6	23.4	8.83	0.86	1.1	0.56	7.79	49
C7	12.6	4.28	0.16	64.3	0.55	3.61	53

1) and 3-d-lagged ($t - 3$) sampling dates, suggesting that tests for significance may have been insufficiently conservative for that stream.

Residual Variation

Despite the relatively good agreement between the observed and predicted (Eq. 2) [DP] in the streams, some uncertainty remains (Table 5). The standard error of predicted DP values ranges between 1.2 and 10.1 $\mu g \cdot L^{-1}$, which is still substantially larger than analytical error (≈ 0.5 $\mu g \cdot L^{-1}$). To explore this further, we examined the residuals (RES; observed minus predicted) of the temperature-dependent model (Eq. 2; Table 5) as time series in each stream to detect any seasonal trend in the performance of the model. No marked tendencies were evident except in C5, where the magnitude of the residuals (both positive and negative) increased toward the end of the sampling period (August). The significance of this is not clear. Nevertheless, this analysis shows that the adequacy of the model remains uniform even in hydrologically rather different periods.

Next we plotted RES as a function of daily solar irradiance, using the latter as an indirect way of assessing the importance of biological (photosynthetic) uptake in affecting DP levels in streams. The irradiance measurements were obtained from the Lake Memphremagog Field Station. The data reflect variation due to cloud cover and to sun angle but do not take into account the phenology of canopy development. In six of the seven streams, there was no significant trend between RES and solar irradiance. In C1, a small nutrient-poor stream, the correlation was significant and negative ($r = -0.45$, $p < 0.01$; removal of the upper leftmost data point in Fig. 7 does not affect this conclusion), indicating that the [DP] on sunny days is lower than that expected from the hydrology–temperature model (Eq. 2) (Fig. 7). With the stream shaded by a dense forest, the pattern observed is probably a consequence of the photosynthetic activity of the forest rather than of any stream alga. However, that the forested catchment C5 did not show an analogous relationship casts some doubts on the generality of the influence of solar irradiance on [DP] in streams.

Influence of Sediment Resuspension

Our modeling efforts have so far neglected the possibility that the soluble phosphorus concentration of stream water is regulated by the stream sediments through sorption/desorption processes, a hypothesis advocated by several authors (Gessner 1960; Meyer 1979; Mayer and Gloss 1980; Hill 1982; Klotz 1985). Much of the supporting evidence is derived from the correspondence between the experimentally derived equilibrium phosphorus concentration (EPC; sensu Taylor and Kunishi 1971) and the average [DP] observed in the streams concerned.

Although we did not directly determine the EPC of the sediment/water systems, aspects of the temporal variation observed in our streams tend to deemphasize the role of sediment chemistry. For example, it is unexpected in the framework of the sediment regulation hypothesis for the [DP] to drop by nearly a half in only a few days, while the concentration of PP remains essentially constant. Such was the case in C5 (July 4, 1984: $Q = 0.8$ $L \cdot s^{-1}$, [DP] = 37.2 $\mu g \cdot L^{-1}$; July 9, 1984: $Q = 172$ $L \cdot s^{-1}$, [DP] = 20.5 $\mu g \cdot L^{-1}$) and similarly in C6 (March 29,

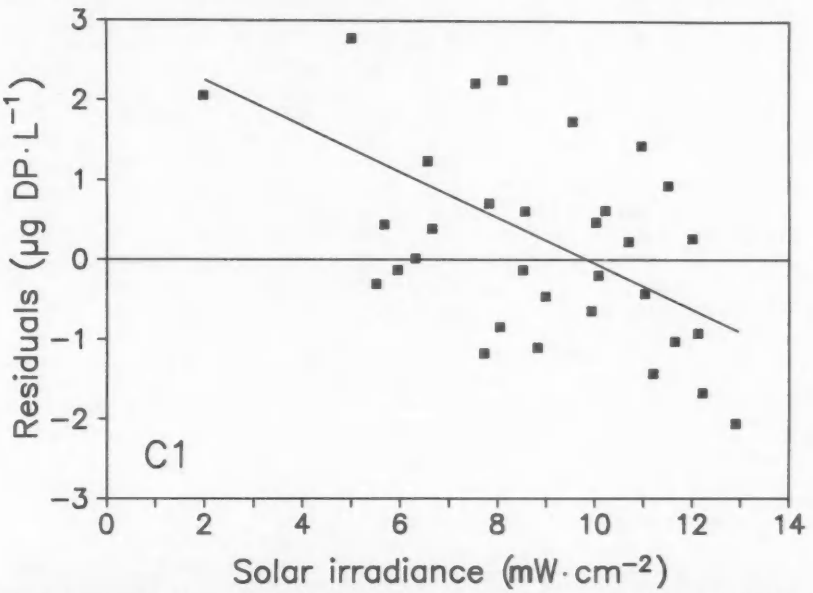

FIG. 7. Scatter plot of the residuals from the temperature-dependent model (Eq. 2; Table 5) in stream C1 versus daily solar irradiance.

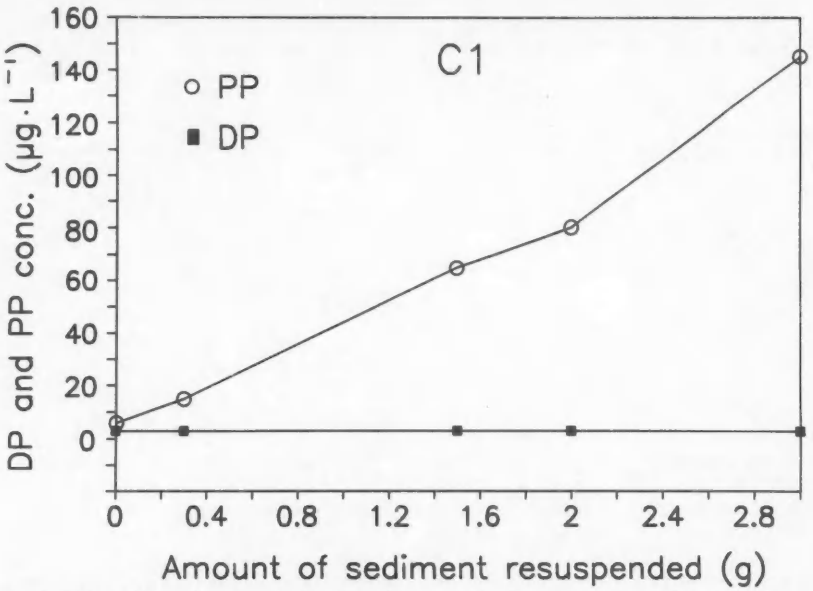

FIG. 8. [DP] and [PP] after 60 min of equilibration time following resuspension of 0.3, 1.5, 2.0, and 3.0 g of fresh sediments in 12 L of C1 stream water.

1984: Q = 54 L·s^{-1}, [DP] = 15.9 µg·L^{-1}; April 2, 1984: Q = 134 L·s^{-1}, [DP] = 8.6 µg·L^{-1}. In both instances, the water discharge increased substantially and probably was responsible for the decrease in [DP].

Although the above evidence is suggestive of the moderate influence of sediment regulation, more convincing support can be derived from the data collected during rain events. While it is conceivable that the dilution (C1,C3,C5,C6, and C7) and the runoff (C2) effects observed in rain events are, respectively, the result of phosphorus sorption and desorption with the storm-induced resuspended sediments, simple laboratory experiments did not support this hypothesis. We spiked 12 L of stream water with varying amounts (0.3, 1.5, 2.0, and 3.0 g; the phosphorus content of the sediments varied between 0.023 and 0.052% by weight of wet sediments depending on the streams) of freshly collected stream sediments under continuous stirring (so as to simulate sediment resuspension) and allowed the solution to equilibrate for 60 min between sediment additions; the [DP] invariably remained constant (within analytical error limits) at the prespiking levels in all streams, despite very large increases in PP (e.g. Fig. 8). This indicates either that the water and sediments were already in equilibrium or that equilibration is a much slower reaction than usually acknowledged (Li et al. 1972; Meyer 1978, p. 126). Alternatively, it may be that the pool of solution phosphorus which actually is in dynamic equilibrium with the sediments is only a small fraction of the total DP. Hence, equilibrium shifts may not be substantial or even detectable in analyzing for DP. Whatever the case, the changes in DP seen during rain events are unlikely to be brought about by sediment resuspension and sorption reactions. Only C4 may be an exception to this rule, since DP there was unrelated to changes in hydrological regime. We conclude that sorption/desorption processes with the stream sediments are unlikely to be the mechanism altering [DP] within the time frame in which the changes in DP do occur during rain events. Nevertheless, such regulation may be operating on a longer time scale. Peters and Cattaneo (1984) also noted a lack of effect of littoral sediment resuspension on lake water soluble phosphorus concentration in Lake Memphremagog.

In summary, our comparison of seven headwater streams has shown that even within one restricted geographical region, catchment land use is not a good predictor of average streamwater [DP]. However, there is a marked tendency for richer streams to be temporally more variable. Part of this seasonal variability can be explained by a simple hydrological model, treating the catchments as two-reservoir systems in which the proportion of water contributed by each reservoir varies with total streamflow. More importantly, this model also encompassed differences in the direction of [DP] – Q relationships observed in our streams and in other studies reported in the literature. The predictive performance of the model was subsequently enhanced by the inclusion of a temperature-dependent parameter. We also note that in a small forested catchment, solar irradiance appears to play a significant role in influencing streamwater concentrations of phosphorus. Finally, laboratory sediment resuspension experiments showed that changes in [DP] are not the result of sediment phosphorus sorption/desorption reactions following sediment resuspension.

Given that our streams cover much of the same variability in average soluble phosphorus levels, seasonal trends, and extent and direction of [DP] – Q relationships that is observed in other streams elsewhere, we hold that our findings are probably not confined to the Lake Memphremagog region but are relevant to streams in general.

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Particulate Phosphorus Dynamics in Headwater Streams[1]

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Prairie, Y. T., and J. Kalff. 1988. Particulate phosphorus dynamics in headwater streams. Can. J. Fish Aquat. Sci. 45: 210–215.

We examined the dynamics of particulate phosphorus (PP) in seven headwater streams of southern Quebec draining catchments differing in size, extent of forest cover, and stream channel gradient. The extent of forest cover can be used to predict average [PP]. The magnitude of the temporal fluctuations (variance) in [PP] was strongly related to mean levels. While most streams showed positive relationships between discharge and [PP] in rain events, inverse relations were observed in two streams and possibly the result of the rapid exhaustion of PP. Inverse relationships were also seen in these two streams for samples collected outside rain events. We present a model based on channel gradient and extent of forest cover to predict the slope coefficient of [PP]–discharge relationships and also the occurrence of very rapid exhaustion. We then extend this model to include the influence of precipitation history and catchment size on [PP] in headwater streams. This model accounted for 60% of the variation of the pooled observations from all seven streams and is comparable in predictive power with stream-specific models published elsewhere in the literature.

Les auteurs ont examiné la dynamique du phosphore particulaire (PP) dans sept cours d'eau d'amont du sud du Québec drainant des bassins versants de superficie, de couvert forestier et de gradient variables. L'importance du couvert forestier peut servir à prédire les [PP] moyennes. L'ampleur des fluctuations temporelles (variance) de [PP] était nettement liée aux niveaux moyens. Tandis que la plupart des cours d'eau montraient des relations positives entre le débit et [PP] en l'occurrence de pluie, on a observé des relations inverses dans deux cours d'eau qui sont peut-être le résultat d'un épuisement rapide du PP. On a aussi noté des relations inverses dans ces deux mêmes cours d'eau dans le cas d'échantillons recueillis après des pluies. Les auteurs présentent un modèle basé sur le gradient du chenal et l'importance du couvert forestier pour prédire le coefficient de la pente de la relation entre [PP] et le débit, et l'occurrence d'un épuisement très rapide. Ils y incluent ensuite l'influence des précipitations et la superficie du bassin versant sur [PP] dans les cours d'eau d'amont. Ce modèle explique 60 % de la variation des observations combinées recueillies dans les sept cours d'eau et est comparable dans sa capacité de prédiction à des modèles particuliers à un cours déjà publiés ailleurs.

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The proportion of phosphorus exported annually from watersheds in the form of particulate material varies between 30 and about 95% and averages about 60% (Reckhow et al. 1980; Y. T. Prairie, unpubl. data). Because lake phosphorus loading models require estimates of the annual load of total phosphorus (TP) (e.g. Vollenweider 1976; Reckhow and Clements 1984; Prairie 1988), the particulate fraction is important, at least numerically. However, the particulate load usually moves only during peak flows (e.g. Meyer and Likens 1979; Munn and Prepas 1986). This makes precise estimation of phosphorus loading difficult because of the need for intensive sampling during periods of highly fluctuating discharge. Errors induced by improper sampling regimes can be large (Stevens and Smith 1978), and several studies (e.g. Johnson 1979; Dolan et al. 1981) emphasize the need for general models that predict phosphorus concentrations at different times and in streams draining catchments of different types. Such general relationships would also serve to integrate the factors influencing phosphorus concentration and help identify new important variables (Likens 1984).

The dynamics of particulate phosphorus (PP) in streams are strongly influenced by catchment characteristics (Hobbie and Likens 1973; Bouldin et al. 1975), water discharge and precipitation (Meyer and Likens 1979; Rigler 1979; Munn and Prepas 1986), chemical composition of suspended sediments (Ongley 1982), and biological transformations within the stream (Meyer and Likens 1979). These important findings are generally the result of intensive studies on a few streams. As a result, their generality is unknown and attempts to integrate the influence of several factors into a general model have been few (Likens 1984).

The principal aim of this study is to develop models predicting the concentration of particulate phosphorus [PP] in a set of diverse streams and at different spatial and temporal scales. The analysis begins by assessing the factors associated with differences in [PP] among streams. We then focus on the

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TABLE 1. General characteristics of the study sites.

Site	Catchment area (km^2)	Forest cover (%)	Channel gradient (%)	Geology
C1	2.0	100	19.2	Igneous
C2	1.5	0	4.1	Sedimentary
C3	2.8	50	2.0	Sedimentary
C4	14.5	75	4.0	Sedimentary
C5	6.3	100	1.4	Sedimentary
C6	4.1	50	3.6	Sedimentary
C7	8.6	67	3.7	Sedimentary/ igneous

detailed temporal changes in [PP] observed during several rain events in the different streams. Lastly, we integrate these factors and the influence of precipitation history into a general empirical model predicting [PP] in headwater streams. This paper is a counterpart to a previous study on the dynamics of dissolved phosphorus in the same streams (Prairie and Kalff 1988).

Study Sites and Methods

The study sites are located in the Lake Memphremagog (45°06′N, 76°17′W) region of Quebec. The area and the sampling methodology have been described in detail in Prairie and Kalff (1988). Briefly, all streams are of first- or second-order catchments varying in extent of forest cover, surface area, and stream channel gradient (Table 1). None of the streams is affected by point sources of phosphorus. The field seasons extended from 1 May to 30 August 1983 and from 20 March to 30 August 1984. We followed two sampling regimes. First, all streams were sampled every 3–4 d on average with additional sampling during times of highly variable discharge (i.e. spring snowmelt). Second, upon the onset of rain, a particular stream was selected and sampled intensively at times of rapidly fluctuating discharge. An average of three rain events were sampled in each stream, with a median sampling time interval of 70 min.

Water discharge was measured at each sampling using the salt (NaCl) injection technique (Church and Kellerhals 1970), which gave a coefficient of variation for replicate runs of less than 10% at low and high discharge and in different streams. Particulate phosphorus concentration was determined as the difference between triplicate samples of TP and total dissolved phosphorus (DP) (0.45-μm washed millipore filter). Samples were analyzed following Strickland and Parsons (1968) after persulfate oxidation and autoclaving (Menzel and Corwin 1965). Since no coarse particulate material (>1 mm) was observed in the samples, the data essentially represent fine PP (Meyer and Likens 1979). The coefficient of variation (percent) due to analytical measurement error can be approximated, for our data, by $72[PP]^{-0.8}$. Since the analytical error variance (σ^2_{PP}) could be calculated for each [PP] estimate, all observations were weighted as $1/\sigma^2_{PP}$ to ensure that resulting statistical models are unbiased.

Daily precipitation at each catchment was estimated by interpolating measurements obtained from three neighboring meteorological stations using the Constrained Optimal Interpolation technique (Rodriguez-Iturbe and Mejia 1974). Tabios and Salas (1985) recently compared several interpolation techniques to rainfall measurements and concluded that this particular technique is amongst the most reliable. Briefly, the method establishes the spatial correlation structure of daily rainfall measurements among all sampled stations and expresses the magnitude of these correlations as a function of the distance between the catchment centroids. Assuming homogeneous variance and isotropic correlation structure, daily precipitation at an unsampled site can be estimated as the sum of weighted precipitations at the sampled stations.

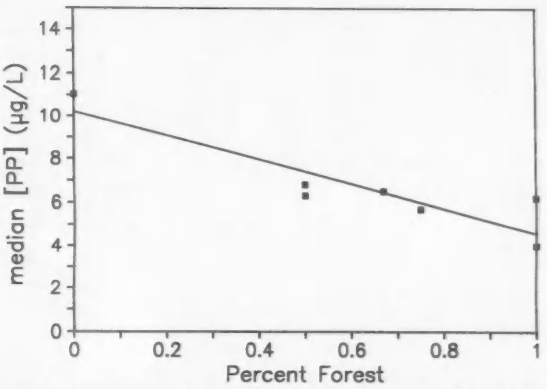

FIG. 1. Relationship between median [PP] and extent of deforestation in the study streams.

Results and Discussion

Temporal Trends and Average PP Levels

Only stream C5 showed a clear seasonal trend: [PP] increased steadily between March and August of both years. The correlation coefficient between [PP] and time (day of the year) for that site was 0.58. Despite the absence of significant temporal trends in all other streams, a correlation matrix of [PP] for matching sampling dates showed that changes in [PP] in one stream were, in general, not reflected in the other streams. Stream C7 was an exception: fluctuations in [PP] in that stream were, albeit to a limited degree, correlated with the [PP] of C1 ($r = 0.39$), C2 ($r = 0.53$), C5 ($r = -0.46$), and C6 ($r = 0.57$).

Although the catchments differ greatly in forest cover, land use, and geology, the mean [PP] are similar, ranging only from 4.5 μg/L in C1 to 15.7 μg/L in C2, with the other streams varying around 10 μg/L. The range of median concentrations is even narrower. Except for C5, the different median values correspond well to the extent of ecosystem disturbance. The coefficient of determination (R^2) between extent of forest cover and mean [PP] is 0.64 and is significant ($p < 0.05$). Using the median concentrations (Fig. 1) explained a greater portion of the variance (80%), although this result is largely dependent on stream C2 (Fig. 1). The regression equation is

$$(1) \quad [PP]_{med} = 10.2 - 0.056 \text{ forest (\%)}.$$

The size of the catchments or the channel gradient was not significantly related to average [PP]. Given that the streams covered the possible range of forest cover but that the range of [PP] observed (4–11 μg/L) was small, we suspect that the model coefficients may apply only to the area of this study.

The [PP] in streams with high mean value is temporally more variable ($\sigma^2_{[PP]} = 0.255[PP]^{2.60}$; $r^2 = 0.92$, $p < 0.0001$). This is also true of DP (Prairie and Kalff 1988).

TABLE 2. Relationships between log-transformed values of [PP] (μg/L) and water discharge (Q, L/s). N is the number of observations, β is the slope of the relationship (log–log), and R^2 is the coefficient of determination.

Site	N	$\beta_{[PP]\text{-}Q}$	$R^2_{[PP]\text{-}Q}$
C1	11	1.21	0.86
C2	33	0.91	0.52
C3	21	−0.14	0.49
C4	28	0.76	0.63
C5	16	−0.69	0.30
C6	10	1.15	0.89
C7	10	0.29	0.50

Changes in Particulate Phosphorus during Rain Events

[PP] – discharge relationships

Published relationships between [PP] and water discharge show hysteresis typical of sediment load: [PP] rises with discharge but the concentrations during the rising stages of the hydrograph are higher than those on the falling stage at equal discharge (e.g. Rigler 1979; Munn and Prepas 1986). Meyer and Likens (1979) divided their data from Bear Brook into season, catchment size, and limb of the hydrograph. Despite this additional refinement, the relationships observed remained weak. Munn and Prepas (1986) demonstrated similar but stronger relationships for two larger Albertan streams. Their data exhibited evident curvilinearity, even after logarithmic transformation of the variables, indicating dramatic increases in concentration at higher discharge. Attempts to juxtapose the data from Bear Brook on those of the Albertan streams showed that no single relationship could characterize all three streams.

In the present study, significant relationships were found in all seven streams between [PP] and discharge for samples collected during rain events (Table 2). To our surprise however, lower [PP] were observed at high discharge in streams C3 and C5. Such different behavior clearly demonstrates that even within a restricted geographical area, changes in [PP] with respect to water discharge cannot be described adequately by a simple concentration–discharge equation for all streams.

Rapid exhaustion of particulate phosphorus

While most streams showed strong positive relationships between [PP] and discharge, streams C3 and C5 exhibited significant negative relationships (Table 2). Since high flows have greater erosive force and sediment carrying capacity, the dilution in these two streams is unexpected and seemingly due to the rapid exhaustion of phosphorus-rich particulate material shortly after the rain begins. Following a very rapid but brief rise in concentration, phosphorus levels declined sharply while discharge was still increasing (Fig. 2). Thus, for the greater portion of the hydrograph, [PP] and water discharge were inversely related. Although PP material is in short supply relative to the stream transport capacity, the total amount of PP transported at high flows is higher than during low flows.

Why was rapid exhaustion restricted to C3 and C5? Both the forest cover and the channel gradient affect the sediment and phosphorus yield of a catchment (Dunne 1978; Wischmeier and Smith 1978; Ahuja et al. 1982). However, in our streams, channel gradient seems to have a larger role in determining sites of rapid exhaustion, with C3 and C5 having the lowest channel gradients (2 and 1.5%, respectively). Although the number of streams we studied is small, our data suggest that a channel gradient of about 3% corresponds to a threshold level below which rapid exhaustion is likely.

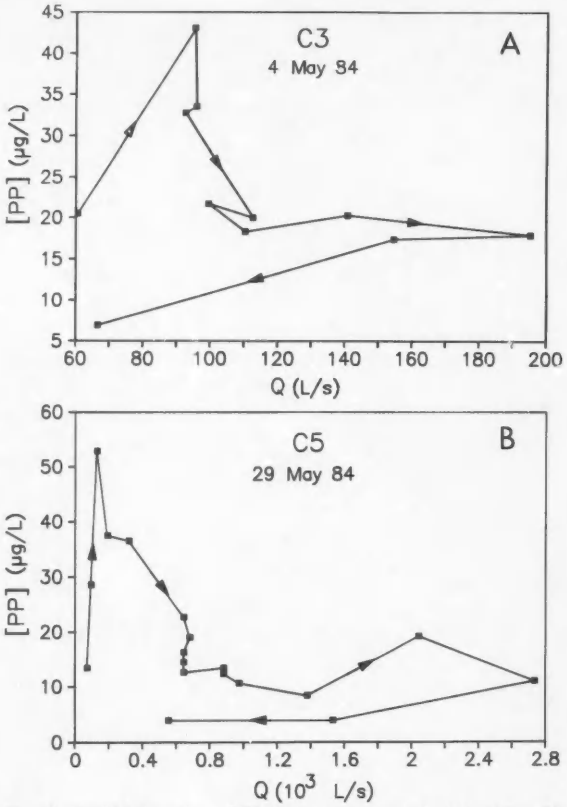

FIG. 2. Rapid exhaustion of PP during rain events in (A) stream C3 and (B) stream C5.

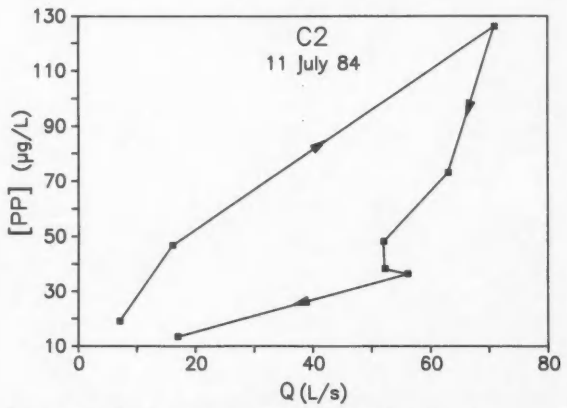

FIG. 3. Relationship between [PP] and Q during the rain event of 11 July 1984 in stream C2 showing clockwise hysteresis. Arrows indicate time sequence.

Stream C2 lies at the other extreme of rapid exhaustion. In this completely deforested catchment, the supply of PP material never seemed depleted. For instance, the moderate rainstorm of 11 July 1984 (~30 mm), showed the usual clockwise loop (Fig. 3), despite the fact that 5 d earlier, a very large rain storm (180 mm in 2 d) occurred. Unfortunately, logistical limitations prevented the assessment of this phenomenon in other streams.

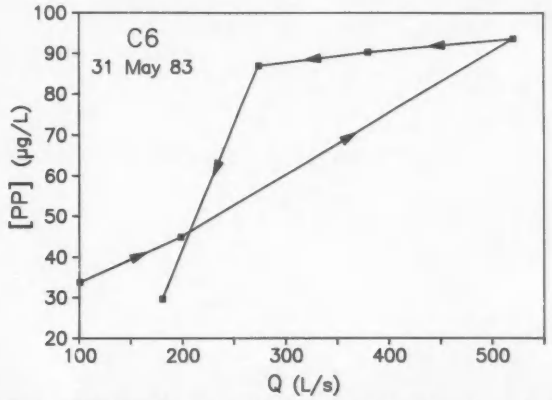

FIG. 4. Relationship between [PP] and Q during the rain event of 31 May 1983 in stream C6 showing anticlockwise hysteresis. Arrows indicate time sequence.

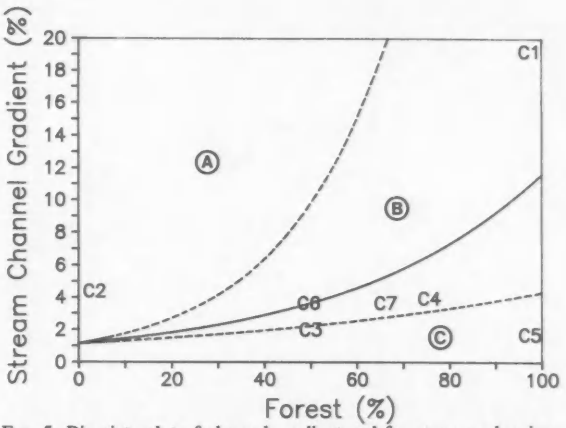

FIG. 5. Bivariate plot of channel gradient and forest cover showing regions where daily [PP]–Q relationships are predicted from Eq. 2 to be positive (A), nonsignificant (B), and negative (C). Broken lines are approximate 95% confidence intervals.

Anticlockwise hysteresis

In most rain events sampled, we observed the usual clockwise hysteresis (Fig. 3). However, the two rain events sampled at stream C6 (31 May 1983 and 15 August 1984) showed reverse hysteresis: [PP] was higher during the receding stage of the hydrograph (Fig. 4). Interestingly, this unusual phenomenon occurred even for events of low magnitude (15 August: ~3 mm of rain). Analogous anticlockwise hysteresis has been observed for dissolved organic carbon (Lesack et al. 1984; T. R. Moore, Department of Geography, McGill University, Montreal, Que., pers. comm.), sodium and TP (Whitfield and Schreier 1981), and suspended sediment (Loughran et al. 1981; Geary 1981 (as reported in Olive and Walker 1982)). The basis for anticlockwise hysteresis is not well understood. For stream C6, the relative position of the forested and nonforested portions within the catchment appears largely responsible. The lower half of the catchment is an undisturbed mixed deciduous forest whereas the upper section is agricultural. Because a disproportionate fraction of the sediments originates from the upper reaches of the catchment, the peak in sediment concentration is necessarily delayed at the sampling site relative to catchments where sites of sediment production are homogeneously distributed. Based on estimates of water velocity in C6, we calculated that at least 60 min is required for sediment particles to travel the total length of the stream channel. This corresponds roughly to the time necessary for the hydrograph peak to actually precede the [PP] maximum.

Changes in Daily Particulate Phosphorus Concentration

The prediction of short-term changes in [PP] in different streams apparently depends on characteristics of the particular stream and catchment. This section considers the prediction of [PP] in terms of mean daily volume-weighted concentration. To this purpose, the samples collected during rain events were used to calculate volume-weighted concentration on those days. These measurements can then be compared with the nonrain data for which the single sampling was assumed to be representative.

Daily [PP] values bore no simple or consistent relationship to discharge. In C3 and C5, where we had observed rapid exhaustion of PP during rain events, daily [PP] was negatively related to discharge ($r = -0.49$ and $r = -0.77$, respectively). In stream C2, where the supply of PP apparently does not limit transport, [PP] and discharge (Q) are positively related ($r = 0.54$). There were no significant trends in the other streams, and in all cases the slope coefficients of the daily [PP]–Q relationships were lower relative to that obtained using the measurements collected during rain events. Such differences between [PP]–Q relationships from samples collected during and outside rain events cast some doubt upon the validity of using storm-derived regression equations between [PP] and Q to estimate long-term transport (e.g. Meyer and Likens 1979).

The dynamics of PP in streams is strongly affected by the combined influence of channel gradient (C3, C5) and extent of forest cover (C2). In statistical terms, these observations imply that the slope coefficient of the [PP]–Q relationships ($\beta_{[PP]\text{-}Q_i}$) in different streams should vary as a function of channel gradient (G) and percent forest (F). Using ordinary least-squares, we obtained the following equation for all our streams:

$$(2)\quad \beta_{[PP]\text{-}Q_i} = -0.02 + 0.38 \log_{10} G - 0.0038F.$$

All regression coefficients were significant ($p < 0.05$) and the overall model explained 80% of the variation. This relationship is more informative as a bivariate plot (Fig. 5) showing the combinations of G and F which yield positive (A), negative (C), and nonsignificant (B) [PP]–Q relationships. Figure 5 shows that negative [PP]–Q relationships (and hence rapid exhaustion) likely occur only in catchments with very shallow channel gradient with little deforestation. Conversely, positive [PP]–Q trends predominate in deforested areas regardless of the channel gradient. Our study does not permit an evaluation of whether, for example, rapid exhaustion occurs in many streams. This question can be addressed using published values of gradient – forest cover combinations. To this purpose, we randomly selected 100 catchments from Omernik (1977) and found that 36 fell into group A, 59 into group B, and only 5 into group C. Our conclusion is that streams exhibiting rapid exhaustion is a relatively rare phenomenon.

Integration of Factors: A General Predictive Model

Particulate phosphorus dynamics are influenced by many factors: average [PP] is correlated with extent of forest cover;

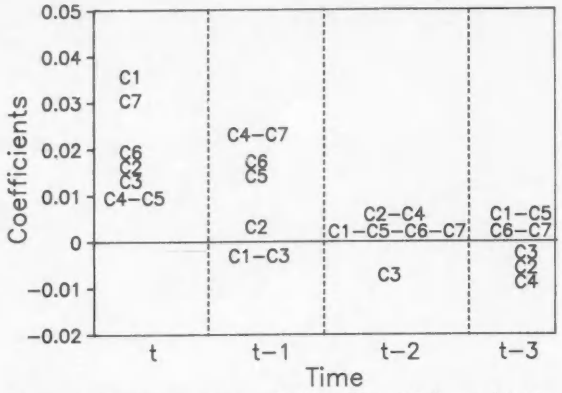

FIG. 6. Distributed lag between [PP] (μg/L) and daily precipitation (mm) in the seven streams.

TABLE 3. Statistics of the multiple regression model of log [PP] on extent of forest cover (F, %), channel gradient (G, %), and their respective interaction terms with water discharge (Q, L/s). $R^2 = 0.51$, $N = 385$, $SE_y = 0.23$.

Variable	Estimate	SE	Prob. > |T|
Intercept	1.353	0.041	0.0001
F	0.272	0.046	0.0001
log G	−0.811	0.067	0.0001
log Q·log G	0.414	0.045	0.0001
log Q·F	−0.407	0.027	0.0001

the position of different land uses within the catchment determines the relative timing of concentration and discharge peaks during rain events; light rainfall can reduce [PP] substantially; discharge interacts with the channel gradient (rapid exhaustion) and extent of forest cover (erodibility). Combination of these factors into a general model is a complex task and one that necessarily sacrifices descriptive precision for predictive generality.

We first performed a multiple regression analysis of the pooled observations ($N = 385$) using four terms: percent forest, channel gradient, and their respective interactions with discharge. All terms were highly significant (Table 3) and the model explained 50% of the variance in [PP]. The coefficients indicate once again that shallow channel gradient will result in a negative influence of discharge on concentration, except in largely deforested watersheds. Because concentration–discharge relationships vary so widely among catchments, the sensitivity of phosphorus transport calculations to hydrological fluctuations will depend largely on extent of forest cover and channel gradient (Meyer et al. 1981).

Next we examined the additional influence of precipitation history on [PP]. The additional independent variables in a model predicting [PP] on day t ($[PP]_t$) were thus daily precipitation on day t ($Prec_t$), $t - 1$, $t - 2$, etc., depending on the length of precipitation history considered. In this analysis, each regression coefficient is a direct measure of the influence of the associated precipitation on [PP]. This approach of fitting a distributed lag between [PP] and precipitation by directly using the precipitation measurements instead of via some polynomial approximation (as is common in econometrics, e.g. Almon 1965; Dhrymes 1971; Judge et al. 1980) is justified on the basis of short length of the lag which we considered (maximum 4 d).

TABLE 4. Statistics of the multiple regression model of log [PP] on extent of deforestation (F, %), channel gradient (G, %), water discharge (Q, L/s), catchment size (CS, km²), daily precipitation variables (Prec, mm), and interaction terms. $R^2 = 0.59$, $N = 384$, $SE_y = 0.21$.

Variable	Estsimate	SE	Prob. > |T|
Intercept	1.304	0.040	0.0001
F	0.162	0.049	0.0010
log G	−0.644	0.071	0.0001
log Q·log G	0.316	0.047	0.0001
log Q·F	−0.315	0.029	0.0001
$Prec_t$	0.012	0.002	0.0001
CS·$Prec_{t-1}$	0.001	0.000	0.0001
CS	−0.009	0.004	0.0256

Nevertheless, this analysis assumes that the influence of the precipitation on each day is the same in all streams. Consequently, we first examined the validity of this assumption by performing an analysis of covariance which considered the homogeneity of the influence of daily precipitation in the different streams. Figure 6 shows the resulting "distributed lags" for each stream.

The first feature to note in Fig. 6 is that the coefficients for days $t - 2$ and $t - 3$ were not significantly different from zero in all streams, indicating that the effective length of precipitation history (in terms of their influence on [PP]) extends for only 1 d for such small catchments. Secondly, the coefficients of $Prec_t$ tended to cluster around the value of 0.016 for all streams except C1 and C7 although their large standard errors encompassed the cluster. Applying a constant value for all streams seems justified in this case, since no serious loss of information is incurred. In contrast, the influence of $Prec_{t-1}$ on [PP] varied sustantially between the streams. The largest (C4) had the highest coefficient and the two smallest catchments (C1 and C2) had nonsignificant coefficients, indicating that the influence of $Prec_{t-1}$ increases with catchment size (CS).

Statistically, the influence of precipitation history on [PP] can thus be translated into (i) a constant coefficient for the $Prec_t$ term and (ii) an interaction term between $Prec_{t-1}$ and catchment size. We thus performed a multiple regression analysis using, in addition to the four variables of the first model (Table 3), the two relevant precipitation history variables ($Prec_t$ and CS·$Prec_{t-1}$). In addition, the effect of catchment area was examined, since it has previously been shown to influence phosphorus export (Prairie and Kalff 1986).

The resulting model (Table 4) explained about 60% of the variation in [PP]. All regression coefficients were highly significant except for catchment area which was only marginally significant ($p = 0.03$). The model includes variables that are constant within each streams, and as a result, their significance may be inflated by the large number of observations within each streams (Hurlbert 1984). However, when we recalculated the partial-F probabilities of these variables using the degrees of freedom associated with the numbers of catchments instead of the total number of observations, the variables were, except for catchment size, still significantly different from zero ($p < 0.05$), confirming the importance of channel gradient and forest cover. The effect of discharge on [PP] was already incorporated in the first model (Table 3). As a result, the significance of the additional precipitation variables (Table 4) represents, statistically, the influence of precipitation on [PP] over and above its effect on discharge. This is probably the result of the effect of precipitation magnitude on the quantity of sediment particles

released and transported in overland flow from the stream bank areas unwashed by the streamflow. The remaining error variance, once back-transformed to arithmetic units, corresponds to 95% confidence limits of about 40 and 250% of the mean. We concede that the uncertainty of this general model is still substantial and that it should be further tested elsewhere but it is comparable with the level of precision of stream-specific models (e.g. Meyer and Likens 1979; Rigler 1979; Munn and Prepas 1986). Our model thus represents an improvement in generality without undue loss in precision.

Acknowledgements

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Influence of Lake Morphometry on the Response of Submerged Macrophytes to Sediment Fertilization[1]

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Duarte, C. M., and J. Kalff. 1988. Influence of lake morphometry on the response of submerged macrophytes to sediment fertilization. Can. J. Fish. Aquat. Sci. 45: 216–221.

The influence of lake morphometry on the growth of submerged macrophytes in response to sediment fertilization was studied at sites of varying bottom slope (steep to gentle), wave exposure (high to low), and depth (1.0 and 2.5 m) in Lake Memphremagog (Quebec–Vermont). The biomass increases were on average 2.1 times greater for fertilized plants than for the paired controls. The extent of the growth response was greatest at the 1.0-m sites. The smaller response at the 2.5-m sites was further decreased as exposure to waves increased. The smaller response at 2.5 m (depth of maximum biomass) suggests that factors such as light limitation, length of the growing season, and littoral slope, but not sediment nutrient levels, limit submerged biomass there. The importance of the physical factors appears to increase as the biomass increases. "Overwintering" plants showed greater response to fertilization than those growing from seeds. Overall, the extent of the growth response to sediment fertilization depends on the energy environment (i.e. depth, wave exposure, and slope) of the littoral. The influence of these physical factors explains previous difficulties in finding strong correlations between nutrient levels and the growth of submerged macrophytes.

On a étudié l'influence de la morphométrie lacustre sur la croissance de macrophytes submergés par rapport à la fertilisation des sédiments à des sites montrant des variations de pentes (abruptes à faibles), d'exposition aux vagues (élevée à faible) et de profondeur (1,0 à 2,5 m) dans le lac Memphremagog (Québec–Vermont). Les augmentations de la biomasse étaient en moyenne 2,1 fois plus élevées chez les plantes fertilisées que chez les plantes témoins. La croissance était plus marquée aux sites de 1,0 m de profondeur tandis que le plus faible taux de croissance aux sites de 2,5 m de profondeur a été davantage diminué en fonction d'une plus grande exposition aux vagues. Le faible taux observé à 2,5 m de profondeur (soit la profondeur de la biomasse maximum) porte à croire que des facteurs comme la lumière, la longueur de la saison de croissance et la pente du littoral, et non les niveaux de bioéléments dans les sédiments, limitent le niveau de la biomasse submergée à ces endroits. L'importance des facteurs physiques semble augmenter en fonction d'une augmentation de la biomasse. Les plantes qui survivent l'hiver montrent une plus grande réaction à la fertilisation que celles qui proviennent de graines. En général, l'amplitude de la réaction de croissance à la fertilisation des sédiments dépend de facteurs environnementaux (p. ex. profondeur, exposition aux vagues et pente) du littoral. L'influence de ces facteurs physiques explique les problèmes antérieurs encourus dans l'identification de corrélations marquées entre les teneurs en bioéléments et la croissance de macrophytes submergés.

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The importance of the biomass and production of submerged macrophytes varies greatly among lakes (Wetzel and Hough 1973). Because the biomass of submerged plants is normally lower in oligotrophic than in eutrophic lakes (e.g. Hutchinson 1975) and because summer phytoplankton biomass correlates well with water nutrient levels, there is a widespread belief that macrophyte biomass too should be a function of nutrient availability. However, the lower biomass of submerged macrophytes in many oligotrophic lakes may be primarily a function of lake morphometry, rather than of nutrient levels (Duarte and Kalff 1986; Duarte et al. 1986). Increased nutrient levels can, in fact, reduce macrophyte biomass by increasing algal biomass, thereby reducing underwater irradiance (Moss 1976; Mulligan et al. 1976; Twilley et al. 1985).

Rooted submerged macrophytes derive most of their nutrient supply from the sediments (Bristow 1975; Nichols and Keeney 1976; Carignan and Kalff 1980; Mantai and Newton 1982). However, attempts to demonstrate a relationship between submerged biomass and sediment nutrient levels have been largely inconclusive (e.g. Anderson 1978; Langeland 1982; Duarte and Kalff 1986). Sediment enrichment experiments in a Canadian lake yielded only modest increases in biomass (Anderson and Kalff 1986), although more substantial increases in biomass following sediment fertilization have been shown in an estuary (Orth 1977). The current literature points to the importance of wave action (Jupp and Spence 1977) and littoral slope (Duarte and Kalff 1986) in determining submerged macrophyte biomass.

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The objective of this study was to resolve the paradox that submerged macrophytes appear at best weakly related to sediment nutrients in oligotrophic and mesotrophic lakes, even though the phytoplankton is strongly nutrient limited. At least two explanations are possible for this paradox: (1) submerged macrophytes in oligotrophic or mesotrophic lakes are not nutrient limited because they can satisfy their nutrient demand from the sediments or (2) the strong influence of the energy environment of their habitat (determined by the wave action, littoral slope, and depth) on submerged macrophytes masks nutrient deficiency. To resolve the issue, we selected sites of different energy environments (i.e. differing in wave action, littoral slope, and depth) in Lake Memphremagog (Quebec–Vermont) and fertilized the sediments to quantify the growth response of the natural plant assemblages.

Methods

The experiment was conducted in Lake Memphremagog (Quebec–Vermont), a large oligotrophic to mesotrophic lake which is sufficiently diverse morphometrically to provide a wide range of exposures and littoral slopes (Fig. 1) (Duarte and Kalff 1986). We tested the effect of the energy environment on the response of submerged plants to sediment nutrient enrichment by fertilizing sites exposed and protected from wave action, with steep and gentle slopes at both 1.0 and 2.5 m so as to consider the decrease in wave energy with increasing depth. The two depths were selected to represent the upper rooting depth in the lake (0.9 m) at which wave action is most severe and the depth of maximum submerged biomass (2.6 m).

To account for the normal 0.5-m decrease in water level over the summer the 0.25-m^2 experimental plots were initially established 0.5 m deeper than the depths reported here. Four experimental quadrats were matched with four control quadrats at each of the two depths at 16 sites (Fig. 1), representing four replicated habitats for each combination of slope and exposure (high or low), yielding a total of 256 plots. At each site, the littoral slope (percent) was measured with an echosounder (Sitex-Honda, model HE-356b) and the exposure to waves calculated in two ways: (1) following a modified weighted effective fetch method (Duarte and Kalff 1986) with the wind frequency and speed obtained from a lakeside meteorological station (E. Petticrew, Biology Department, McGill University, Montreal, Que., unpubl. data) and (2) as the area of water exposed to each site, or effective area (E_{area}, square kilometres), measured from a 1 : 34 130 map with a digitizer (Hi-Pad, Houston Instruments). The second method was used because the shape of Lake Memphremagog does deflect the wave direc-

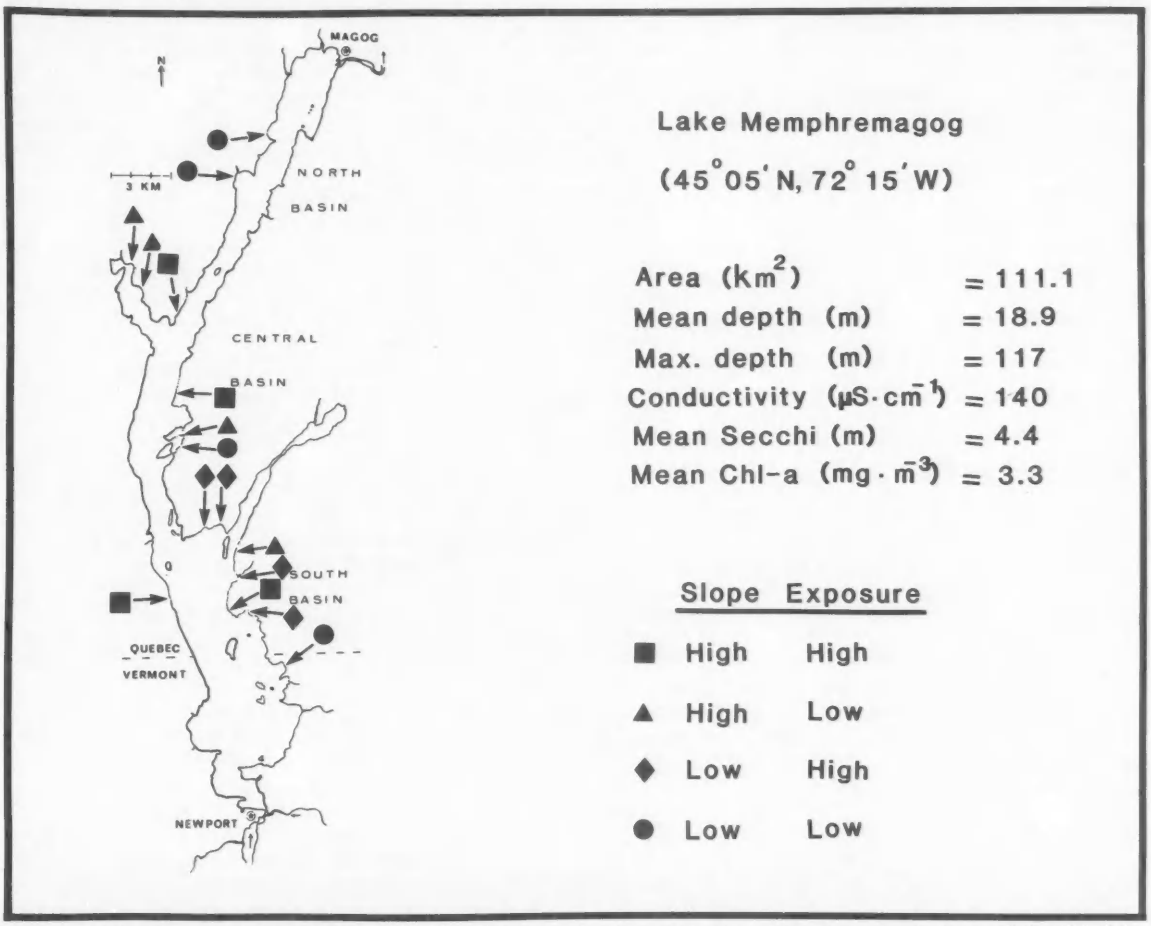

FIG. 1. Lake Memphremagog showing the sites of the four different treatments and the main morphometric and water characteristics of the lake.

TABLE 1. Environmental characteristics of the experimental sites and sediment covariates, showing the differences between the high (H) and low (L) treatments and the shallow (1.0 m) and deep (2.5 m) treatments. All variables differ significantly at the two levels ($P < 0.05$). E_{area} = effective area; TP = sediment total phosphorus; WC = sediment water content; OM = sediment organic matter.

Variable	Level	Mean	SE
E_{area} (km²)	H	15.9	1.5
	L	3.0	0.6
Slope (%)	H	7.5	0.4
	L	2.0	0.08
TP (mg·g dry wt^{-1})	1.0 m	0.52	0.04
	2.5 m	0.87	0.04
WC (% fresh wt)	1.0 m	32.4	0.91
	2.5 m	47.3	1.37
OM (% dry wt)	1.0 m	2.53	0.24
	2.5 m	10.33	0.74
Sediment depth (cm)	1.0 m	21	12
	2.5 m	132	33
Secchi disk (m)	All	4.5	0.4

tion by up to 60° from the prevailing wind direction. Therefore, wind direction measured at the lakeside metereological station is a poor indicator of wave direction at individual sites. The water transparency at the 16 sites was measured three times during the course of the experiment using a 20-cm Secchi disk.

Unlike previous in situ sediment manipulations (Anderson and Kalff 1987; Chambers and Kalff 1985), we used the natural plant community and sediments. We attempted to minimize the variability introduced by local habitat heterogeneity by using replicated paired plots (treatment versus control) at each site. The four fertilized plots at each depth were placed 9 m apart and the control plots at 3 m from the corresponding fertilized one. The individual plots were marked with styrofoam floats 0.5 m above the sediments. SCUBA divers fertilized the experimental plots between June 6 and 9 with a 15-cm-long spike of 200 g of slow-release fertilizer containing nitrogen, phosphorus, and potassium with a ratio 3:1:1. The plants were then either still dormant or just starting to emerge (surface water temperature 14°C). When possible, the fertilizer spikes were pushed to a depth of 15 cm in the sediments, thereby encompassing the depth of the greatest root density in Lake Memphremagog (C. M. Duarte, unpubl. data). A second spike was placed within 10 cm of the first on July 15 or 16, when the first was still present, so as to guarantee a continuing supply of nutrients. Nutrient supply to the plants in the plots was adequate, since the long vascular root systems of the species involved (ranging from 7 to 10 cm in diameter for *Najas flexilis* R. to almost 100 cm in *Myriophyllum spicatum*. L.) are very efficient in long-range (20–30 cm) nutrient transport (Bottomley and Bayly 1984; Mantai and Newton 1982). Furthermore, Orth (1977), who used a fertilization technique very similar to ours, reported that responses to fertilization were visually evident at distances well beyond the area covered by our plots.

The growth response of the plants to fertilization was measured as the biomass increase over the paired control. As production of submerged macrophytes often exceeds biomass increases by about 50% (Rich et al. 1971), our measurement of growth underestimates the increase in plant production because it does not account for biomass loses. The experimental sites were harvested, using SCUBA, just prior to the attainment of maximum biomass so as to minimize natural plant mortality. All of the aboveground parts of the submerged plants rooted

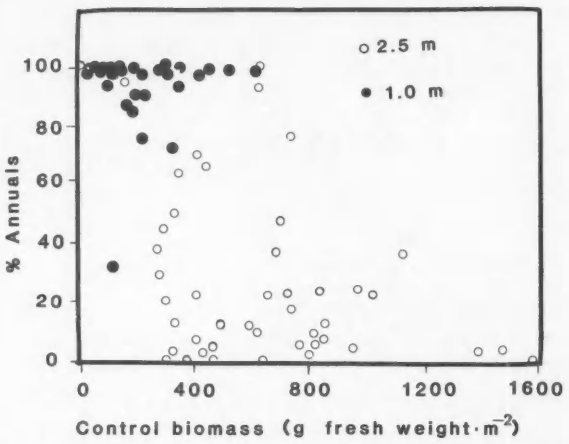

FIG. 2. Proportional contribution of "annual" plants to the control biomass as a function of the control biomass for shallow and deep plots.

inside the quadrats were collected and then refrigerated until processed. The plants were rinsed free of detritus and animals and those species in which aboveground biomass disappears overwinter, here referred to as "annuals" (mostly *Vallisneria americana* M., *Najas flexilis*, and *Heteranthera dubia* M.), were separated from "overwintering plants" (largely *Myriophyllum spicatum, Potamogeton praelongus* W., and *Potamogeton robinsii* O.). Both classes were weighed to the nearest 0.01 g after excess water had been removed with a lettuce spinner.

Just prior to harvesting, a sediment core was taken with a Plexiglas corer (5 cm internal diameter) to a depth of 10 cm to determine water content, organic matter content, and total phosphorus concentration of the sediment. Sediment depth was measured at four random locations within each quadrat using a metal rod. Sediment water content was measured to represent sediment conditions (Hakanson 1977, 1981; Duarte and Kalff 1986). The sediment was extruded into a container and frozen within 3 h of collection for subsequent analysis. At that time, the sediments were first mixed and then the water content was determined after drying overnight at 110°C. Organic content was determined from the mass lost after overnight ignition at 540°C. Analysis of total phosphorus followed Andersen (1976).

Due to the disappearance of 24 markers, only 241 of the 256 plots were recovered at the end of the experiment. Because of the nature of the experiment, the slope and fetch could not be exactly duplicated at the four replicate sites (Table 1). We used an analysis of covariance to remove the effects of uncontrolled variables (sediment water content, organic matter, phosphorus concentrations, and water transparency) and to account for variations in the control variables (slope and wave exposure) within treatments (Steel and Torrie 1960).

Results and Discussion

Characteristics of Control Plots

The mean slope, exposure, and sediment depth values of the different sites are presented in Table 1. Sediments at 1.0 m were thinner and coarser, with lower water content, lower organic content, and lower phosphorus concentration than those at 2.5 m. The marked differences in sediment characteristics at the

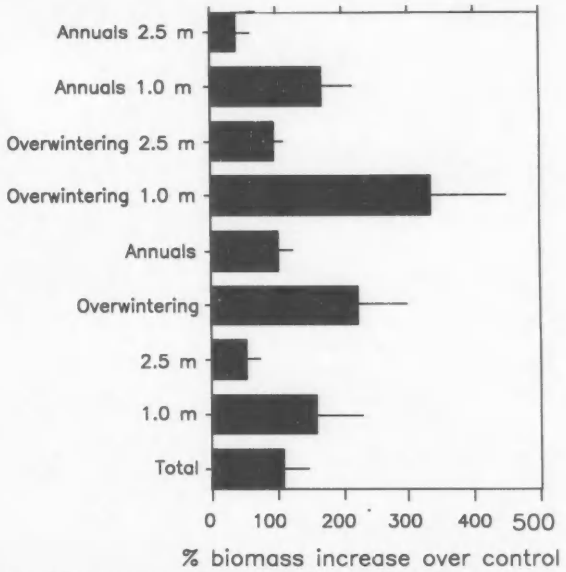

FIG. 3. Average percent biomass increase in the fertilized plots over the paired controls ((experimental biomass − control biomass)/control biomass) for all plots, the two experimental depths separated, for "overwintering" and "annual" plants, and for their response at the two depths. The bars represent 1 SE.

two depths reflect the greater effect of wave action and ice scouring in shallower waters. Lake water transparency decreased slightly from south to north ($r = 0.34$), the average Secchi disk transparencies for the 16 sites ranging from 3.9 to 5.1 m.

No plants were found in 12 of the 128 control plots at the start of the experiment, but only 7 of these, all shallow exposed quadrats, lacked vegetation by the end of the experiment. The biomass in the control plots, therefore, ranged from 0 in some steep and in shallow exposed sites to 1745 g fresh wt·m^{-2} at a deep and gently sloped site. The biomass of the control plots was higher in the 2.5-m plots than in the shallow areas (t-test, $P < 0.0001$), and at the greater depth it was higher on the gently sloped sites (t-test, $P < 0.0001$). The "overwintering" plants (*Myriophyllum spicatum*, *Potamogeton praelongus*, and *Potamogeton robinsii*) dominated the biomass in the deep plots whereas the "annual" species (*Vallisneria americana* and *Najas flexilis*) were dominant in the shallow ones (Fig. 2) (Wilcoxon ranked sign test, $P < 0.001$).

Influence of Nutrient Additions on Biomass

The biomass in the fertilized plots was on average more than twofold greater than that in the paired controls (Fig. 3) (Wilcoxon ranked sign test, $P < 0.0001$). This growth response is greater than reported for previous in situ fertilization experiments in this lake (Anderson and Kalff 1986). The present strong responses obtained may be the result of the use of (1) large amounts of slow-release fertilizer and (2) natural communities that avoid confounding factors such as transplanting stress, space problems due to confinement in containers, differential rooting success, or competition with the existing plant communities. Furthermore, results obtained from transplants of a single species may well not be extrapolatable to natural communities adapted to the particular conditions in each plot.

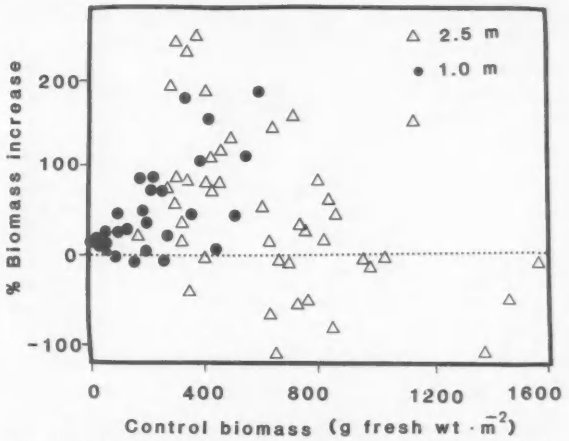

FIG. 4. Relationship between the percent biomass increase over the control plots as a function of the control biomass for the 1.0- and the 2.5-m plots.

Overwintering plants responded more strongly to nutrient addition than annual plants (Fig. 3) (Wilcoxon ranked sign test, $P < 0.0001$), indicating that established vegetation used the added nutrients more than did the annuals. This is not surprising because the overwintering plants establish both root and photosynthetic systems earlier in the growing season than the annual plants do, allowing them to use sediment nutrients earlier in the season. The greater response of overwintering plants (Fig. 3) suggests that transplant experiments could underestimate the effect of fertilization because they force all species to behave like annuals (Chambers and Kalff 1985; Anderson and Kalff 1987). At deeper sites, the annual plants did not respond significantly ($P > 0.05$) to fertilization, while overwintering plants responded to fertilization in both shallow and deep plots, but with a greater biomass increase in the shallow sites (Fig. 3).

Morphometric Influences on Biomass Response to Fertilization

There was a significant response to fertilization in all habitats (t-test, $P < 0.001$) except for the plants growing at 2.5-m plots with low fetch and steep slope (t-test, $P > 0.05$). Sites well protected from wave action should experience an at best small sediment resuspension and, therefore, experience low losses of sediments and associated nutrients. However, at the same time, steep slopes are an unsuitable environment for plant growth (Duarte and Kalff 1986). The specific reasons are unclear but probably are multifactorial because slope affects sediment thickness, grain size, and stability (Hakanson 1977, 1981) as well as the dissipation of wave energy. The littoral slope also influences the horizontal extent of weedbed, possibly because plants growing on steep slopes will reach the depth of light compensation much closer to shore than would be the case with gentle slopes. The resulting narrower weedbeds will, therefore, be much more exposed to disturbance. Because the response to fertilization varied considerably among sites (Fig. 3), we tested whether this variability could be partially attributed to differences in littoral slope and exposure to waves. We examined the influence of these morphometric factors on the biomass increase using regression analysis. The wave environment of each site was measured as the effective area (E_{area}) because it is better related to sediment texture, as reflected in the water content

($r = 0.62$), than is the weighted effective fetch ($r = 0.53$). The experimental depth was represented as a categorical variable taking the values 1 (2.5 m) and 0 (1.0 m).

The analysis of covariance of the increase in growth showed that increasing exposure to wave action and slope significantly reduced the growth response to fertilization when the two depths were considered together. However, since the wave action for a given site is more severe at 1.0 m than at 2.5 m, the nature of its influence on the response to fertilization probably differs at the two depths. Furthermore, the control biomass was positively related to the biomass increase in the fertilized plots at 1.0 m but negatively related at 2.5 m (Fig. 4). Consequently, the growth responses of deep and shallow stands were analyzed separately.

The analysis for the 2.5-m depth explained 32% of the variation in growth increase (experimental biomass − control biomass, grams fresh weight per 0.25 m²) and showed that the response to nutrients decreased with increasing exposure and with increasing control biomass:

(1) Growth increase = 214 − 7.7(10.4) E_{area} − 0.35(7.4) control biomass, $r^2 = 0.32$, EMS = 5488, $N = 49$

where the numbers in parentheses are the partial F values for the different variables and EMS is the error mean square.

The experimental depth of 2.5 m is relatively large compared with the wave height (between 0 and 0.5 m in Lake Memphremagog). Therefore, the negative effect of exposure on the growth response (Eq. 1) may be the result of a direct physical stress on the plants because the canopy here often extends close to the surface. Despite the strong relationship between littoral slope and control biomass at 2.5 m (Duarte and Kalff 1986), the response to nutrient additions there is not dependent on the littoral slope (F test, $P > 0.05$). This supports evidence presented by Duarte and Kalff (1986) that the relationship between slope and maximum submerged biomass is independent of nutrient levels. No other variables (sediment thickness, water content, organic content, phosphorus concentration, and water transparency) contributed significantly to explain variability in the growth response of the plants. The negative relationship between control biomass and growth response (Eg. 1) indicates that plants growing in sites characterized by a high biomass are not nutrient limited. These plants are therefore limited by other factors that might include the length of the growing season or self-shading (light limitation) and/or the littoral slope.

The growth response at shallow sites was proportional to the control biomass ($r^2 = 0.50$), and no effect of morphometric factors was found:

(2) Growth increase = 33.9 + 0.65(47.7) control biomass, $r^2 = 0.50$, EMS = 917, $N = 56$.

The analysis shows that nutrient limitation here is the principal factor controlling submerged growth at 1.0 m. This is supported by the positive relationship between growth increase and control biomass at this depth (Fig. 4). This relationship would not have been positive if nutrient limitation had been absent at the sites characterized by the most favorable growing conditions (i.e. highest control biomass). Coarser sediments, lower levels of sediment total phosphorus and organic matter (Table 1), and higher light levels in the shallower sites accentuate the importance of nutrient limitation there. The marked response of shallow-water plants to nutrient additions (Fig. 3) indicates the importance of erosion of sediments and associated nutrients by the combined effects of wave action (Hakanson 1977) and ice scouring, reducing the effective nutrient levels in these areas. Therefore, it appears that severe nutrient limitation at 1.0 m exceeds the direct physical stress resulting from exposure to waves and currents. The absence of a direct relationship between plant response to fertilization and exposure to waves suggests that spring ice scouring, which is relatively homogeneous around the lake shore, has a greater influence on sediment erosion than wave action. Although the amount of nutrients supplied was the same at all sites, the dynamics may have differed depending on the wave intensity, sediment grain size, and slope at the different locations. The resulting lack of precise control over the treatment variables among sites, together with the variability in species composition, must be responsible for the large unexplained variance in growth response (Eq. 1 and 2), an error that would have been smaller had we studied plant growth under controlled conditions. In addition, any vegetation loss prior to harvesting will result in underestimation of the real extent of nutrient limitation and add to the unexplained variance in growth response.

General Discussion and Conclusions

Nutrient limitation of submerged macrophytes growth was demonstrated. However, the extent of this limitation differed between the shallow and deeper sites and varied with wave exposure and the natural biomass of the littoral zone. In contrast with the idea that direct physical disturbance by wave action controls macrophyte biomass in the littoral zone (Jupp and Spence 1977; Spence 1982), our results implicate nutrient limitation as the proximal regulating factor. It appears that wave action at the shallow sites leads to the erosion of fine-grain particles and associated nutrients, enhancing nutrient limitation at these sites. On the other hand, the direct physical stress produced by wave action on the plants, together with the degree of slope and perhaps light levels, appears to contribute to the regulation of growth at the depth of maximum biomass (2.5 m).

The field sites in this study represent wide ranges of wave exposure, littoral slope, and sediment characteristics. Thus the results presented here should be applicable to other oligotrophic or mesotrophic lakes. Extrapolation from our experiment suggests that the macrophytes of large lakes with high wave action and steep slopes should respond less to sediment nutrient enrichment at a depth of 2.5 m than those at 1.0 m in smaller lakes or well-protected bays with similar light levels. On the other hand the growth response at 1.0 m is not influenced by wave exposure or slope. The extent to which macrophyte communities respond to fertilization is not only a function of lake morphology, but also of whether they are dominated by "annual" or by "overwintering" plants. The annual plants appear to be less influenced by sediment nutrient levels than the overwintering ones (Fig. 3). Annual plants also reach a much smaller maximum biomass (Fig. 2), probably because they are more constrained by the length of the growing season than by nutrient limitation.

The lake littoral is a zone of great complexity, where the different physical and biological factors interact to produce very different responses to the same disturbance. The difficulties in demonstrating sediment nutrient limitation in submerged macrophyte communities appear attributable to the array of interacting environmental conditions and floristic characteristics that determine the extent of nutrient limitation.

Acknowledgements

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Influence of Molecular Weight and Molecular Volume on Dietary Absorption Efficiency of Chemicals by Fishes

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Rainbow trout (*Salmo gairdneri*) were exposed to 17 halogenated organic chemicals to examine the influence of molecular weight (MW) and molecular volume (MV) on dietary absorption efficiency. The results of this and other studies indicated that organic chemicals with MWs greater than 600 were poorly absorbed although some chemicals with lower MWs were also poorly absorbed. A better trend was suggested when efficiences were examined in relation to MV where chemicals with MVs over 0.3 nm^3 were poorly absorbed. Neither index provided a relationship that would consistently estimate the absorption efficiency of chemicals that were moderately to well absorbed by salmonids.

Des truites arc-en-ciel (*Salmo gairdneri*) ont été exposées à 17 produits chimiques organiques halogénés afin d'examiner l'influence du poids moléculaire (PM) et du volume moléculaire (VM) sur l'efficacité d'absorption des aliments. Les résultats de la présente expérience et d'autres études révèlent que les produits chimiques organiques dont le PM est supérieur à 600 sont faiblement absorbés quoique certains produits chimiques à faible PM sont aussi mal absorbés. On a obtenu une meilleure tendance quand les efficacités ont été étudiées en fonction du VM dans les cas où les produits chimiques à VM supérieur à 0,3 nm^3 étaient mal absorbés. Aucun des indices n'a généré une relation qui pourrait estimer uniformément l'efficacité de l'absorption de produits chimiques modérément absorbés ou bien absorbés par les salmonidés.

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Contaminant dynamics models often include a coefficient to describe the uptake efficiency of a chemical. Relatively few studies have reported this coefficient for fishes because data on uptake and elimination rates are needed to estimate this value (Neely 1979; Niimi 1983). Water exposure studies indicate absorption efficiences of 0.03–0.6 for chemicals whose log octanol/water partition coefficients (K_{ow}) range from 0.2 to 9.2 (Neely 1979; Boese 1984; McKim et al. 1985). Dietary exposure studies indicate efficiences of 0.6–0.9 for chemicals such as polychlorinated biphenyls (Tanabe et al. 1982; Niimi and Oliver 1983). Other workers report that chemicals such as hexabromobenzene, octachlorodibenzodioxin, and tetradecachloroterphenyl were poorly absorbed by fish exposed to dietary levels of 4–50 $mg \cdot kg^{-1}$ for 40–70 d (Zitko and Hutzinger 1976; Bruggeman et al. 1984). Comparable studies on mammals also indicate that chemicals such as paraffins and polystyrenes are not absorbed after an oral dose (Albro and Fishbein 1970; Monte 1983). These observations indicate that absorption efficiences among chemicals can differ, and physico-chemical properties may influence their uptake efficiency.

Previous studies have indicated that chemicals with molecular weights greater than 600 were poorly absorbed by fish, and molecular size could be a contributing factor (Zitko 1974; Tulp and Hutzinger 1978; Bruggeman et al. 1984). These observations were generally reported in the course of studies that were not specifically conducted to examine this factor. Molecular size can be an important variable because the relationship between volume and weight of a chemical will differ by severalfold depending on elemental composition and structural arrangement. For example, iodobenzene (C_6H_5I) and *n*-tetradecane ($C_{14}H_{30}$) have similar molecular weights although the latter is twice the volume of the former, while *n*-dodecane ($C_{12}H_{26}$) and hexabromobenzene (C_6Br_6) are similar in volume based on their parachor values (Quayle 1953) although the latter is over three times heavier than the former.

This study examined the dietary uptake of selected planar and nonplanar halogenated organic chemicals by rainbow trout (*Salmo gairdneri*) to determine the extent to which molecular weight (MW) and molecular volume (MV) can influence

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absorption efficiency. These results were compared with estimates from other studies to assess some factors that may influence chemical absorption by fishes.

Materials and Methods

Hatchery-reared trout, about 300 g each, were held in 250-L tanks according to conditions described by Niimi and Oliver (1983). Four tanks containing 30 fish each were maintained at 13 ± 1°C. All fish were fed about 1% of body weight daily on a dry diet (Martin Feed Mills, Elmira, Ont.).

After 4 wk of acclimation, fish were fed an oil mixture that contained a group of chemicals. The chemicals were dissolved in herring oil according to the procedure described by Niimi and Oliver (1983). One chemical group included 1,2,4-tribromobenzene, 1,3,5-tribromobenzene, 1,2,4,5-tetrabromobenzene, 1,2,3,4-tetrachloronaphthalene, hexachlorobutadiene, and hexachlorobenzene (HCB). The second group contained γ-chlordane, *p,p'*-DDE, mirex, octachloronaphthalene, octachlorostyrene, 2,2',4',5-tetrabromobiphenyl, 2,2',4,5',6-pentabromobiphenyl, 2,2',4,4',6,6'-hexabromobiphenyl, and HCB. The third group included pentabromotoluene, pentabromoethylbenzene, hexabromobenzene, and HCB. A fourth oil preparation was the control which contained only HCB that was used as an internal standard for all groups. These chemical standards were obtained from Aldrich Chem. Co., Milwaukee, WI; Chem Serv. Inc., West Chester, PA; ICN K&K Laboratories, Plainsview, NY; Ultra Sci., Hope, RI; and Dow Chem. Co., Walnut Grove, CA.

Each group of fish was given a single oral dose that contained one of three chemical groups or the control preparation. Each fish was anesthetised with 100 mg tricaine methanesulfonate·L^{-1} and, than a gelatin capsule containing 0.5 mL of the oil mixture was inserted into the stomach with a glass tube. All fish were fed several hours after exposure and on a daily basis thereafter. Fish were exposed to 54–66 μg of each chemical (Table 1). Six fish from each tank were sampled 5, 14, 28, 48, and 85 d after exposure. All fish were frozen at −20°C until analyses. Whole-fish homogenates were prepared for chemical analyses according to procedures described by Niimi and Oliver (1983).

Samples were prepared for analyses using the clean-up procedures described by Oliver and Nicol (1982). Chemical levels were determined by electron-capture gas chromatography. Analysis of fish exposed to the first two groups of chemicals and the control group was done using an instrument equipped with a split/splitless injection port with the splitless valve on for 30 s. A DBS or a DB1 30 m × 0.25 mm ID capillary column was used. Column temperature was programmed at 50–280°C at 2°·min^{-1} with a final hold of 40 min. Injection port and detector temperatures were 250 and 350°C, respectively. Detector makeup gas was nitrogen at 30 mL·min^{-1}, and carrier gas was helium at 2 mL·min^{-1}. Fish exposed to the third chemical group were analysed on an instrument with an on-column injection port. An OV1 25 m × 0.31 mm ID column was used. Injection port was at ambient temperature, column temperature increased from 70 to 310°C at 10°·min^{-1} with a final hold of 10 min, and detector temperature was 320°C. Detector makeup gas was methane–argon (5 + 95) at 30 mL·min^{-1}, and carrier gas was hydrogen at 1.8 mL·min^{-1}. Percent recoveries of spiked samples for most chemicals were generally in the 80–90% range. Lipid levels were also determined following the method of Bligh and Dyer (1959). Values for all chemicals were expressed as micrograms per fish and lipid as percent weight on a wet weight basis.

Absorption efficiency (AE) was calculated by dividing the amount of chemical in fish at the beginning of the study by the amount fed. Initial chemical level in fish at day 0 was estimated

TABLE 1. Chemicals of different molecular weights (MW) and corresponding molecular volumes (MV) that were fed and retained by trout after 5–85 d. Values shown represent the mean ± SD of six fish, except those identified with an asterisk where $n = 5$. Absorption efficiency (AE) and whole-body half-life in days ($T_{1/2}$) are shown for each chemical.

Chemical	MW	MV	Amount fed (μg)	Amount retained (μg) 5 d	14 d	28 d	48 d	85 d	AE	$T_{1/2}$
Tetrachloronapthalene	266	0.19	54	16±3	8±2	4±1	2±1	2±1	0.44–0.21[a]	8–27[a]
Octachloronaphthalene	404	0.25	60	9±2	6±1	4±2	4±2	3±1	0.18–0.11[a]	15–62[a]
Hexachlorobutadiene	261	0.14	66	20±3	12±2	9±2	2±1	2±1	0.38–0.27[a]	14–23[a]
Pentabromotoluene	487	0.20	58	15±8	8±3	5±5*	1±1	1±1	0.28–0.18[a]	13–23[a]
1,2,4-Tribromobenzene	315	0.14	61	44±8	30±6	21±4	7±2	3±1	0.88–0.79[a]	16–20[a]
1,3,5-Tribromobenzene	315	0.14	56	32±6	22±2	16±2	5±2	3±1	0.68–0.59[a]	18–22[a]
Tetrabromobenzene	394	0.16	54	24±5	12±2	9±7	2±1	1±1	0.66–0.42[a]	9–16[a]
Hexabromobenzene	551	0.21	54	14±8	7±2	3±1*	3±1	2±1	0.28–0.17[a]	>13–31[a]
Pentabromoethylbenzene	501	0.22	56	24±5	12±2	9±7*	6±1	3±1	0.26	38
Tetrabromobiphenyl	470	0.23	55	37±3	36±2	33±3	30±1	27±2	0.68	161
Pentabromobiphenyl	548	0.25	58	40±1	41±4	38±4	38±1	37±2	0.69	>300
Hexabromobiphenyl	627	0.28	57	30±1	32±3	29±3	30±2	30±2	0.54	>300
Octachlorostyrene	380	0.23	56	37±2	38±2	38±2	36±2	35±2	0.68	>300
γ-Chlordane	339	0.23	54	32±2	30±1	28±4	27±2	22±2	0.60	147
p,p'-DDE	318	0.24	59	43±7	37±6	43±7	43±2	45±4	0.67	>300
Mirex	546	0.30	56	27±2	28±2	28±2	26±2	27±1	0.48	>300
Hexachlorobenzene										
I	285	0.18	63	48±7	43±2	41±2	45±4	37±3	0.73	297
II			63	50±6	52±4	46±5	46±3	29±2	0.88	102
III			63	45±9	44±6	39±6*	37±9	30±6	0.73	133
IV			63	50±5*	41±2	48±3	37±7	35±7	0.77	167
Mean									0.78	175

[a]Estimates based on 5- and 14-d sample interval and 5- to 85-d interval.

by interpolation from coefficient A of the relationship $Y = Ae^{bt}$ based on residue levels monitored in fish during the study (Niimi 1986). A whole-body half-life for each chemical was estimated from the regression coefficient b of the same relationship.

AE was related to the MW and MV of each chemical. MV (cubic nanometres per molecule) was estimated from the coefficients of individual and functional groups of elements based on van der Waals radii (Bondi 1964). Parachor was also used to estimate volume for comparative purposes. Parachor is a number calculated from the atomic parachor value for each element and structural value for the elemental arrangement of each compound (Sugden 1930). Estimates of MV by either method for nonplanar compounds such as mirex should be viewed as approximate because their "three-dimensional" configuration may not always allow calculations to be made following the procedure used for planar compounds such as HCB.

Results and Discussion

Absorption Efficiency and Half-Life Estimates

One control fish sampled after 5 d and another after 28 d after receiving the third group of chemicals contained 6–10 μg of HCB, while levels among other fish ranged from 36 to 60 μg. This would suggest that most of the oral dose administered to these two fish was regurgitated; therefore, these results were not considered. Fish weight increased an average of 125 g and lipid from 6.7 to 11.4% during the 85-d study. Mean values of 9–50 μg of each chemical were present in fish after 5 d of exposure even though they were fed similar amounts (Table 1).

Levels of some chemicals such as tetrachloronaphthalene (TCNP) declined rapidly, while others such as mirex remained similar during the study. AEs ranged from 0.11 for octachloronaphthalene to 0.79 for 1,2,4-tribromobenzene based on chemical levels monitored in fish over 85 d (Table 1). AEs for the HCB internal standard were 0.77 for the control group and averaged 0.78 among the three experimental groups.

Low initial chemical level, a rapid decline to levels approaching analytical detection limits, and minimal changes at these levels are factors that can underestimate AE and overestimate the half-life of a chemical. For example, the AE for TCNP based on levels in fish 5 and 14 d after exposure was 0.44 and was 0.21 based on levels between 5 and 85 d. Half-life estimates of TCNP for the same sample intervals were 8 and 27 d, respectively. In both cases, the differences in values were due to the influence of the low TCNP levels observed between 28 and 85 d which reduced the interpolated coefficients of A and b. This concentration-dependent response was also suggested for other chemicals with short biological half-lives that we examined (Table 1). This factor should not influence AE or half-life estimates where chemical levels are high even though changes in levels during the observation period may be small because of the principle of superposition (Levy and Gibaldi 1975). According to this principle, the response between any two sample intervals should be similar to that estimated using all the sample intervals (see fig. 2 of Niimi 1983). The half-life values estimated in this study are in good agreement with values previously reported for some chemicals (Niimi 1987).

Molecular Weight and Volume Uptake Efficiency

Dietary absorption efficiency generally declined with increasing MW among the chemicals examined whose estimates were not influenced by their half-lives, although this relationship was not well defined (Fig. 1). To provide a broader basis for examining this response, dietary AEs reported by other studies were also considered (Table 2). The relationship

$$AE = \frac{C_f \, Ke}{C_d \, (1 - e^{-Ket})}$$

can be used to calculate AE where C_f is the chemical content of fish at time t, Ke is the half-life or clearance coefficient, and C_d is the chemical content of food. This equation is particularly useful for long-term studies on chemicals with short half-lives to account for the amount that had been eliminated during the study. Equations to calculate AE have been reported by others although a prolonged exposure period would be required to attain chemical equilibrium (Bruggeman et al. 1981). In a few studies where feeding rates were not reported (e.g. Zitko 1980),

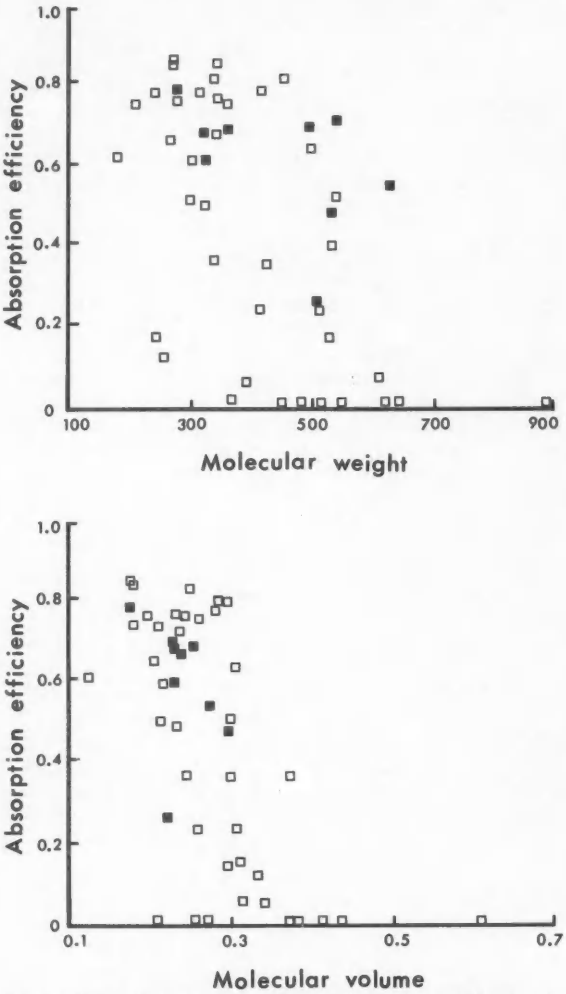

FIG. 1. Dietary absorption efficiences of chemicals for salmonids in relation to molecular weight and molecular volume. Solid squares represent some values observed for trout in this study reported in Table 1, and open squares represent the values for salmonids estimated from other studies in Table 2.

TABLE 2. Dietary absorption efficiences (AE) of chemicals reported for fishes in relation to molecular weight (MW) and molecular volume (MV). Values in parentheses were estimated from the data presented.

Chemical	MW	MV	AE	Species	Ref.[c]
Chlorinated biphenyls (PCBs)					
Dichloro-	223	0.18	0.74[a]	Trout	1
			0.89[a]	Carp	2
Trichloro-	258	0.20	0.77[a]	Trout	1
			0.87[a]	Carp	2
Tetrachloro-	292	0.21	0.74[a]	Trout	1
			0.86[a]	Carp	2
			(0.44)	Guppy	3
Pentachloro-	326	0.23	0.78[a]	Trout	1
			0.84[a]	Carp	2
Hexachloro-	361	0.24	0.76[a]	Trout	1
			0.82[a]	Carp	2
			(0.61)	Guppy	3
Heptachloro-	396	0.26	0.76[a]	Carp	2
Octachloro-	430	0.28	0.78	Trout	1
			0.72	Carp	2
			(0.47)	Guppy	3
Nonachloro-	465	0.29	0.80	Trout	1
Decachloro-	499	0.31	0.63	Trout	1
			(0.27)	Guppy	3
Aroclor 1254	350	0.23	0.68	Trout	4
Chlorinated diphenyl ethers					
Trichloro-	273	0.20	0.65	Trout	5
Tetrachloro-	308	0.22	0.60[a]	Trout	5
Pentachloro-	342	0.23	0.49[a]	Trout	5
Hexachloro-	377	0.25	0.36[a]	Trout	5
Heptachloro-	412	0.26	0.24[a]	Trout	5
Decachloro-	515	0.31	0.24	Trout	5
Chlorinated dioxins					
Tetrachloro-	306	0.21	(0.50)	Trout	6
Octachloro-	444	0.28	(0.01)	Guppy	3
Chlorinated furans					
Octachloro-	428	0.27	(low)	Salmon	7
Trichlorobenzene	182	0.13	0.61	Trout	8
Pentachlorobenzene	250	0.16	(0.40)	Guppy	9
Hexachlorobenzene	285	0.18	0.85	Trout	10
			0.84[b]	Trout	11
Tribromobenzene	315	0.14	(0.45)	Guppy	9
Tetrabromobenzene	394	0.16	(0.09)	Guppy	9
Pentabromobenzene	472	0.18	(0.06)	Guppy	9
Hexabromobenzene	551	0.21	(0.01)	Guppy	9
			(nil)	Salmon	12
Pentabromotoluene	487	0.20	(0.02)	Guppy	11
Chlordane	339	0.23	0.34	Sucker	13
Methoxychlor	346	0.28	0.80	Trout	14
DDT	354	0.25	(0.83)[b]	Trout	15
			0.62[b]	Menhaden	16
Dieldrin	381	0.25	(0.73)[b]	Trout	15
			(0.73)	Catfish	17
Oxytetracycline	460	0.38	(0.01)	Trout	18
Kepone	491	0.27	(0.31)[b]	Spot	19
			(0.24)	Catfish	20
Mirex	546	0.30	0.52[b]	Trout	11
			(0.37)	Trout	21
			(0.15)	Salmon	22
Dechlorane 604	509	0.27	(0.01)	Salmon	22
602	614	0.34	(0.06)	Salmon	22
603	638	0.38	(0.01)	Salmon	22
Plus 25	654	0.41	(0.01)	Salmon	22
Tetradecachloroterphenyl	713	0.44	(0.01)	Guppy	3
Alkanes					
Dodecylcyclohexane	252	0.31	(0.18)	Trout	23
Pristane	269	0.33	(0.12)	Trout	23
Chlorowax 500C	411	0.32	(>0.06)	Trout	24
Squalane	423	0.37	0.38	Trout	25
Cereclor 42	489	0.44	(nil)	Salmon	26
Chlorez 700	903	0.61	(nil)	Salmon	26

[a]Mean of several homologues.

[b]Mean of several treatments.

[c]References: 1, Niimi and Oliver (1983); 2, Tanabe et al. (1982); 3, Bruggeman et al. (1984); 4, Lieb et al. (1974); 5, Niimi (1986); 6, Kleeman et al. (1986); 7, Zitko and Choi (1973); 8, Smith et al. (1980); 9, Bruggeman (1983); 10, Niimi and Cho (1980); 11, Niimi and Palazzo (1985); 12, Zitko and Hutzinger (1976); 13, Roberts et al. (1977); 14, Kruzynski and Leduc (1972); 15, Macek et al. (1970); 16, Warlen et al. (1977); 17, Argyle et al. (1975); 18, Herman et al. (1969); 19, Stehlik and Merriner (1983); 20, van Veld et al. (1984); 21, Skea et al. (1981); 22, Zitko (1980); 23, Cravedi and Tulliez (1982); 24, Lombardo et al. (1975); 25, Cravedi and Tulliez (1986); 26, Zitko (1974).

the amount of chemical fed using a dry diet was estimated from a feed to gain ratio of 1.3 based on the increase in fish weight during the study (Cho et al. 1976).

Some differences in AEs between species were suggested among the values reported in Table 2. Estimates for female guppies (*Poecilia reticulata*) were generally lower than comparable values reported for salmonids. No other comparative analyses of AEs among species would be feasible at this time because of limited data. No general relationship between MW and dietary AE among salmonids could be demonstrated from the cumulative data (Fig. 1).

The relationship between AE and MV indicates a trend that is better defined than one with MW (Fig. 1). Chemicals with a MV of 0.25 nm^3 or less tended to be absorbed at an AE of 0.6–0.8. AEs then decreased for chemicals with a MV of 0.25–0.3 nm^3 and remained low thereafter for those with nonlinear configurations. It cannot be determined if the relationship between AE and chemicals of less than 0.4 nm^3 is linear or if the AE for most chemicals of less than 0.25 nm^3 is in the 0.6–0.8 range, declining thereafter as MV increases.

Transport Across Cell Membranes

Several hypotheses have been proposed on the transport of chemicals across cell membranes. Aqueous pores may facilitate movement of highly polar organic chemicals of low MV across membranes (Yalkowsky and Morozowich 1980). Other studies suggest that pore size may not be important in regulating chemical movement across biological membranes although diffusion rates were shown to be inversely related to molecular mass (Lieb and Stein 1969). The fluid mosaic model of cell membrane structure proposes a bimolecular phospholipid layer where hydrophilic compounds are absorbed by active transport, while hydrophobic compounds pass through the membrane by diffusion (Singer and Nicolson 1972). These membrane characteristics suggest that AE efficiency may be influenced by sev-

eral variables which respond to different properties of the chemical.

Chemical speciation is a major consideration in cellular transport. The chemicals examined in this study were non-ionic organic compounds. AEs of waterborne phenolics and carboxylic acids are strongly influenced by their dissociation constants (Saarikoski et al. 1986). Other studies indicate that organic mercury was absorbed at AEs of 0.2 from water and 0.8 from food, while inorganic mercury was absorbed at AEs of 0.02 and 0.2 from water and food by rainbow trout (Hamelink et al. 1977; Boudou and Ribeyre 1985). Radioelements such as ^{60}Co and ^{241}Am were also poorly absorbed through diet, with AEs of 0.007–0.06 (Kimura and Ichikawa 1972; Carvalho et al. 1983).

Estimates of Chemical Size

It is difficult to apply one measurement of the physical dimension of a chemical that could be consistently applied to many organic chemicals found in aquatic ecosystems. Differences in structural arrangements among the more persistent chemicals is one of the major factors. MVs based on van der Waals radii were derived from planar chemicals of low MW, while the composition of the more environmentally relevant organic chemicals can be nonplanar and of high MW. Parachor can account for structural components in the third dimension although its value is a dimensionless number. For example, HCB has a MV of 0.18 nm^3 and a parachor value of 430. A good linear relationship between MV based on van der Waals radii and parachor is indicated based on the 54 chemicals listed in Tables 1 and 2 with a correlation coefficient (r^2) of 0.94. Similar linear correlations among the same chemicals between MV and van der Waal volume gave an r^2 value of 0.55 and between molecular weight and parachor gave an r^2 value of 0.54. The relationship between AE and MV (Fig. 1) was not enhanced when efficiency was plotted against parachor.

Molecular "shape" is another factor that could influence AE. Limited information indicates that chemicals with linear configurations such *n*-alkanes can be absorbed at a higher efficiency than nonlinear chemicals with similar volumes. For example, squalane with a MV of 0.37 nm^3 was absorbed at an AE of 0.4 which was much higher than other chemicals with similar volumes (Fig. 1). Alkanes may "bend" at intervals of seven to eight carbons presenting a configuration that is helical rather than linear, thereby reducing the actual chemical size (Wall et al. 1970).

Size as a Limiting Factor

Dietary and waterborne AEs for a chemical can differ, as indicated earlier for mercury. Dietary AEs of 0.8 for HCB and 0.5 for mirex were observed in this study, while waterborne AEs of about 0.6 and 0.2 were observed in rainbow trout (McKim et al. 1985). The shape of the AE response may also differ between dietary and waterborne uptake. AEs of 0.07–0.6 were reported for 14 waterborne chemicals by trout (McKim et al. 1985). These values follow a bell-shaped curve when plotted against their MVs which range from 0.07 to 0.3 nm^3. A similar-shaped curve was indicated by English sole (*Parophyrys vetulus*) for four phthalates with MVs of 0.2–0.4 nm^3 whose AEs ranged from 0.03 to 0.4 (Boese 1984). A reexamination of the data of Neely (1979) indicated a linear increase in waterborne AEs among seven chemicals whose MVs range from 0.09 to 0.2 nm^3. Saarikoski et al. (1986) reported a linear increase in AEs for 10 chemicals with MVs of 0.09–0.15 nm^3, and then no changes in efficiences of six chemicals with MVs up to 0.2 nm^3. Neither of the last two studies examined chemicals whose volumes approached those in the first two studies where waterborne AEs decreased to corroborate the bell-shaped curve.

Our study indicates that MW or MV alone is not sufficient to explain the differences in AEs although these indices are consistent with the physiological basis of nutrient absorption by animals. Food products that are ingested are subject to enzymatic action before absorption. Polysaccharides are reduced to monosaccharides, proteins to amino acids, and lipids to fatty acids. A representative sugar such as glucose has a MW of 180 and MV of 0.1 nm^3 and the 20 alpha-amino acids have MWs of 75–204 and MVs of 0.07–0.18 nm^3, while glycerin and stearic acid have MWs of 92 and 284 and MVs of 0.08 and 0.33 nm^3, respectively. These values are well within the range where the highest AEs were observed in fish, even for stearic acid with its linear configuration. Dietary AEs of 0.90–0.96 have been reported for protein nitrogen, lipid, and energy content of food by fish (Beamish 1972). These values are only slightly higher than the coefficients reported for chemicals of comparable MV (Fig. 1).

Indices such as MW, parachor, and molecular surface area have been correlated with other physical parameters such as water solubility and K_{ow} (Briggs 1981; Doucette and Andren 1987). Other studies on fishes have reported good correlations between MW and bioconcentration factor (BCF) and parachor and BCF although these studies were limited to chemicals with MVs below 0.3 nm^3 (Kanazawa 1982; Tulp and Hutzinger 1978; Oliver and Niimi 1984). No consistent trends were indicated when AEs were related to chemicals with high K_{ow} values. A relationship of decreasing efficiences with increasing K_{ow} was suggested among the chlorinated diphenyl ethers and low AEs among hydrophobic chemicals such as dechloranes (Table 2). This relationship was not supported by the chlorinated biphenyls where AEs remained at the 0.7 range for trout and carp even though K_{ow} values for dichloro- to decachlorobiphenyls, calculated by the π method of Hansch and Leo (1979), ranged from 5.5 to 11.2. The more hydrophobic chemicals examined in this study such as the brominated biphenyls with K_{ow} values of 7.5–9.2 and AEs of 0.6 would further suggest that chemicals with high K_{ow} values can have high or low dietary AEs.

It is obvious that further studies are required to examine factors that influence chemical absorption by fishes. Based on this discussion, we postulate that AE could be described by several indices that reflect biological as well as a chemical processes.

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Chemical Fractionation and Bioavailability of Cobalt-60 to Benthic Deposit-Feeders

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Evans, R. D., D. Andrews, and R. J. Cornett. 1988. Chemical fractionation and bioavailability of cobalt-60 to benthic deposit-feeders. Can. J. Fish. Aquat. Sci. 45: 228–236.

Concentrations of radiocobalt (^{60}Co) and stable Co were measured in a series of sequential chemical extractions of freshwater sediment cores and in benthic deposit-feeding organisms. In all sediment samples, a large fraction of the stable Co was measured in the strong-acid (residual) extract. Co concentrations in the exchangeable and carbonate-bound fractions decreased with depth in the core, while Co concentrations in the oxide- and organic-bound or sulfide-bound fractions increased. We hypothesize that the changes in Co partitioning result from the decrease in E_h with depth as $FeCO_3$ dissolves and FeS_2 forms. ^{60}Co added to sediments was incorporated quickly by the exchangeable, carbonate-bound, and oxide-bound fractions. Very little ^{60}Co was measured in the organic and residual fractions although these fractions contained more than 50% of the total stable Co. The specific activity of ^{60}Co in organisms (Becquerels ^{60}Co per microgram total Co) that were incubated in sediments spiked with radiocobalt was similar to the specific activity of the exchangeable, carbonate, and oxide fractions. The large percentage of Co in the organic and residual fractions was not accumulated by the benthic deposit-feeders. The concentration factor (ratio of organism concentration to sediment concentration) for radioactive Co was the same as that of stable Co from the first three fractions only, which suggests that there may be a relationship between concentration factors and the bioavailable fraction of a contaminant. Specific activity may provide a simple measure of the bioavailable fraction of substances.

On a quantifié les concentrations de radiocobalt (^{60}Co) et de Co stable au cours d'une série d'extractions chimiques séquentielles de carottes sédimentaires prélevées en eaux douces et chez des organismes détritivores benthiques. Dans tous les échantillons de sédiments, une importante fraction du Co stable a été quantifiée dans l'extrait (résidu) d'acide fort. Les concentrations de Co dans les fractions échangeables et liées au carbonate ont diminué en fonction de la profondeur dans la carotte tandis que les concentrations de Co dans les fractions liées à l'oxyde, aux matières organiques ou au sulfure ont augmenté. Les auteurs formulent l'hypothèse que les variations du fractionnement du Co sont le résultat d'une baisse du E_h selon la profondeur au fur et à mesure que le $FeCO_3$ se dissout et que le FeS_2 se forme. Du ^{60}Co a ajouté à des sédiments a rapidement été incorporé par les fractions échangeables liées au carbonate et à l'oxyde. Les concentrations de ^{60}Co dans les fractions organiques et résiduelles étaient très faibles quoique celles-ci contenaient plus de 50 % de la concentration totale de Co stable. L'activité spécifique du ^{60}Co chez des organismes (Becquerels ^{60}Co par migrogramme de Co total) incubés dans des sédiments additionnés de radiocobalt était semblable à l'activité spécifique des fractions échangeables liées au carbonate et à l'oxyde. Le pourcentage élevé de Co dans les fractions organiques et résiduelles n'a pas été accumulé par les détritivores benthiques. Le facteur de concentration (rapport entre la concentration chez les organismes et la concentration dans les sédiments) du Co radioactif était le même que celui du Co stable dans le cas des trois premières fractions seulement, ce qui porte à croire qu'il existe peut-être une relation entre les facteurs de concentration et la fraction biodisponible d'un élément polluant. L'activité spécifique peut servir de quantification simple de la fraction biodisponible de certaines substances.

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Significant fractions of many trace metals and radionuclides discharged into freshwaters are bound rapidly to particles and transported to the bottom sediments. Once these contaminants are deposited in the sediments, they may be buried permanently, recycled into the water column, or accumulated by benthic feeding biota. The divergent behaviour of the same element in different systems may reflect the accessibility of the contaminant to dissolution reactions or the contaminant's association with particulate materials with different chemical char-

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acteristics. Using selective chemical extraction procedures (Tessier et al. 1979; Luoma and Jenne 1976b; Luoma and Bryan 1978, 1979), pools of trace metals associated with different chemical fractions have been identified. The distribution between fractions is presumed to be controlled by the geochemical conditions in the sediments and by the form in which the contaminant was input originally to the system. These variables also will influence the bioavailability of the contaminants. Studies correlating contaminant concentrations in specific fractions with the geochemistry of the system (Tessier et al. 1980; Evans and Parliament 1984) and with contaminant concentrations in benthic fauna collected from different sites (Luoma and Jenne 1976a; Tessier et al. 1984) support these hypotheses.

Differences in chemical partitioning of a radionuclide and its stable analogues may explain variations in their behaviour and bioavailability. Studies of fate of radionuclides released into aquatic environments often have expressed the biological uptake of these chemicals as concentration factors (ratio of organism concentration to environment concentration) (Ophel 1963; Ophel and Fraser 1971; Ophel et al. 1972; Emery et al. 1975; Edgington et al. 1976). In practice, concentration factors are difficult to interpret because of variability between and within systems (Emery et al. 1975). Specific activity of a radionuclide within various environmental compartments may provide better information on the bioavailability of that substance.

In this paper, we report measurements of ^{60}Co and stable Co concentrations in sediment cores collected from the Ottawa River, in spiked sediment experiments performed in the laboratory, and in benthic deposit-feeders from field and laboratory studies. Using the sequential extraction procedures of Tessier et al. (1979), we have tested the hypotheses that (1) ^{60}Co and stable Co are not found in the same chemical extract, (2) ^{60}Co and Co partitioning changes after the isotopes are put into the sediments and (3) the different ^{60}Co and Co body burdens in benthic deposit-feeders can be explained by the different chemical partitioning of ^{60}Co and Co, (4) ^{60}Co specific activity can be used to identify the chemical fractions that are available to benthic biota, and (5) concentration factors for both stable and radioactive Co will be the same when expressed as a function of the bioavailable pool.

Methods

Chalk River Nuclear Laboratories (CRNL) have discharged low levels of fission and activation products into the Ottawa River for about 40 yr. The discharge emerges about 10°C above the ambient temperature through a subsurface pipe 1 m in diameter. Approximately 1% of the annual flow rate of the river (900 $m^3 \cdot s^{-1}$) is discharged through the effluent. At this location, the river is about 0.75 km wide with a mean depth of 25 m.

Sediment cores were collected in Plexiglas core tubes (6.3 cm I.D.) with a modified K-B corer at a location in the river with a water depth of 20 m, downstream from the effluent pipe from the Chalk River plant. The sediment cores were extruded and sliced at 1-cm intervals under a nitrogen atmosphere in a glove bag. A nitrogen atmosphere was used and maintained during subsequent analyses to prevent oxygen from affecting the chemical partitioning of the metals in sediments (Rapin et al. 1986). After sampling each slice to measure porosity (percent water loss after drying to constant mass at 50°C), organic content (loss on ignition at 450°C), and total activity of ^{60}Co and ^{137}Cs, aliquots (1–3 g) of each sediment slice were

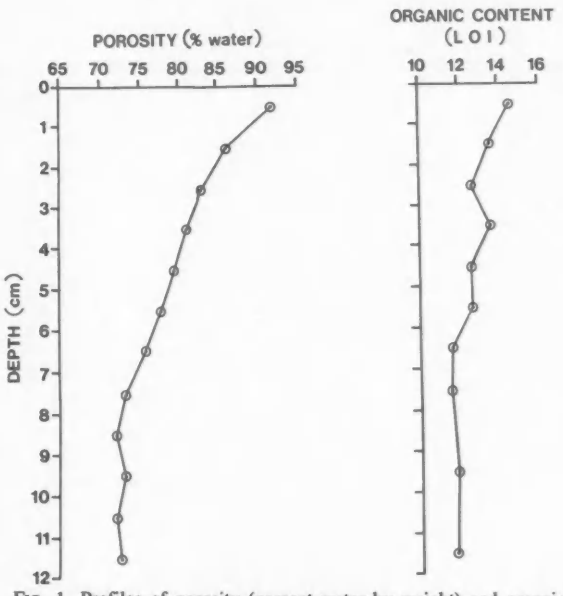

FIG. 1. Profiles of porosity (percent water by weight) and organic content (loss on ignition) in core B.

placed in 50-mL acid-washed polycarbonate centrifuge tubes, capped under nitrogen, and centrifuged to extract the interstitial water. The interstitial water then was poured into acid-washed vials and the centrifuge tubes were flushed and recapped under nitrogen and frozen for later chemical partitioning. Freezing, by halting bacterial action and retarding the diffusion of oxygen into the sediments, is the best method available for storing the samples (Rapin et al. 1986).

Aliquots of each bulk sediment sample (unsieved) were fractionated chemically according to a sequential extraction procedure modified from that of Tessier et al. (1979) into the following operationally defined metal fractions: (1) easily exchangeable (extractable in 1 *M* $MgCl_2$, pH 7), (2) bound to carbonate (extractable in 1 *M* NaOAc at pH 5), (3) bound to iron and manganese oxides (extractable in 0.04 *M* NH_2OH-HCl in 25% HOAc), (4) bound to organic matter (extractable in 30% H_2O_2 at pH 2), and (5) residual (extractable in hot concentrated HNO_3). This extraction procedure differs slightly from that given by Tessier et al. (1979). The first three extractions were performed under a nitrogen atmosphere and the residual fraction was extracted by two digestions in 8 mL of concentrated HNO_3. It was felt that any Co remaining after the two digestions in hot HNO_3 could not be assimilated by benthic organisms. In all extractions, "ultra-grade" reagents were used whenever available. Analyses of each chemical extract for total Co were performed on a Scintrex flameless atomic absorption spectrophotometer following extraction by ammonium pyrrolidine dithiocarbamate (APDC) into methyl isobutyl ketone (MIBK) (Anonymous 1985). The APDC–MIBK extraction procedure was found to be essential to eliminate interferences in some of the chemical fractions. Samples of the chemical extracts were also counted for ^{60}Co and occasionally for ^{137}Cs on a 5.08-cm NaI (T1) scintillation detector coupled to a 2048-channel analyzer.

To examine the short-term binding of ^{60}Co and its subsequent chemical partitioning, surface sediments low in ^{60}C were col-

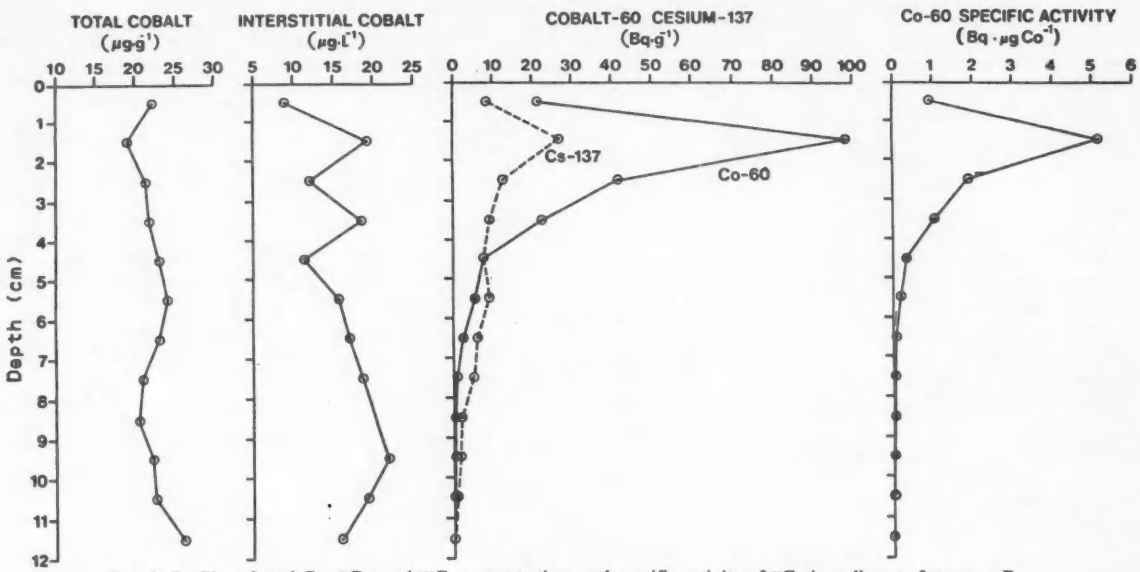

FIG. 2. Profiles of total Co, ^{60}Co, and ^{137}Cs concentrations and specific activity of ^{60}Co in sediments from core B.

lected by coring at a station 1.5 km upstream of the CRNL plant effluent pipe. To 100 mL of sediment was added 1.9×10^4 Bq of ^{60}Co as $CoCl_2$. The radioactive Co added had a negligible effect on the total Co content of the sediment. The sediments were mixed rapidly and sampled over a 15-d period with a 50-mL syringe. Each sediment sample was added to a 50-mL acid-washed polycarbonate centrifuge tube and chemically fractionated as described above. Since the sediments were originally oxygenated, analysis under a nitrogen atmosphere was not considered necessary. The chemical extracts were analyzed for stable Co and ^{60}Co as described above.

Levels of stable Co and ^{60}Co were measured in the natural benthos at the site of core collection near the CRNL effluent. Five Eckman dredge samples were collected, sieved through a 250-mm-mesh nylon net, and picked for macroinvertebrates. After allowing at least 48 h for evacuation of their guts in filtered lake water, the organisms were dried on preweighed strips of acid-washed plastic sheeting, weighed on a microbalance, and then digested with 1 mL of H_2O_2 (80%) or concentrated "ultra-grade" HNO_3 in acid-washed teflon vials or polyethylene centrifuge tubes. No attempt was made to remove metal bound to the surface of the organisms. The residues were reconstituted in 0.1 *N* HNO_3. The extracts were analyzed for stable Co by atomic absorption and for ^{60}Co in a low-background NaI (T1) scintillation detector. Concentrations of Co in blank extractions usually were negligible. For comparison, animals also were collected from Round Lake near Peterborough, Ontario, and analyzed for total Co.

Because population densities of macroinvertebrates near the plant discharge were low, sufficient faunal biomass for an accurate determination of ^{60}Co activity in the fauna at the downstream station could not be obtained. To compensate for the lack of macroinvertebrate biomass, commercially available specimens of the benthic oligochaete *Tubifex* sp. were cultured in these downstream sediments taken from three depth horizons, representing three activities of ^{60}Co (Fig. 2): 0–2 cm (high activity), 3–5 cm (medium activity), and 9–11 cm (low activity). Fifty worms were cultured in each of five replicates of each sediment type in 250-mL glass crystallizing dishes containing about 100 mL of sediments. Three control dishes of sediment without animals were used also. The crystallizing dishes were covered with aerated river water and maintained in the dark at 5°C. Prior to addition of benthos, samples of sediment were taken for chemical partitioning of stable Co and ^{60}Co. At three sampling periods, 20, 50, and 110 d, five animals were removed from each dish and analyzed for stable Co and ^{60}Co as described above. Sediment subsamples were also taken at the conclusion of the experiment for chemical partitioning.

Tubifex specimens also were incubated in duplicate 50-mL aliquots of low-activity (upstream) Ottawa River sediments to which ^{60}Co (1.9×10^4 Bq) as $CoCl_2$ was added. After 3 wk, 50–60 animals were placed in each beaker and cultured for 16 wk at 5°C. Five replicate samples of 10 worms each were taken from each experimental beaker, allowed to evacuate their guts, and analyzed for stable Co and ^{60}Co, and sediment samples were taken to determine the chemical partitioning of stable Co and ^{60}C.

Results and Discussion

Composition of Ottawa River Sediments

The sediments sampled near the CRNL outflow pipe are composed of compacted gray silts and clays with a flocculent, brown oxidized layer in the upper 1–2 cm. There is a steep porosity gradient in the upper 10 cm and the water content falls from 92 to 72% over this interval (Fig. 1). The organic content of the sediments, expressed as a loss of ignition (LOI) at 450°C, is about 11–12% and increases slightly towards the surface to 14.5% in the brown oxidized layer (Fig. 1). The stable Co concentration of the sediments changes little over 12 cm of depth and remains at 22.4 ± 1.8 μg Co·g dry weight^{-1} (Fig. 2). Interstitial concentrations, although more erratic, also fail to show any distinct trend with depth. Expressed per gram, the concentrations of stable Co in the interstitial water are three orders of magnitude less than those in the dried sediment. The

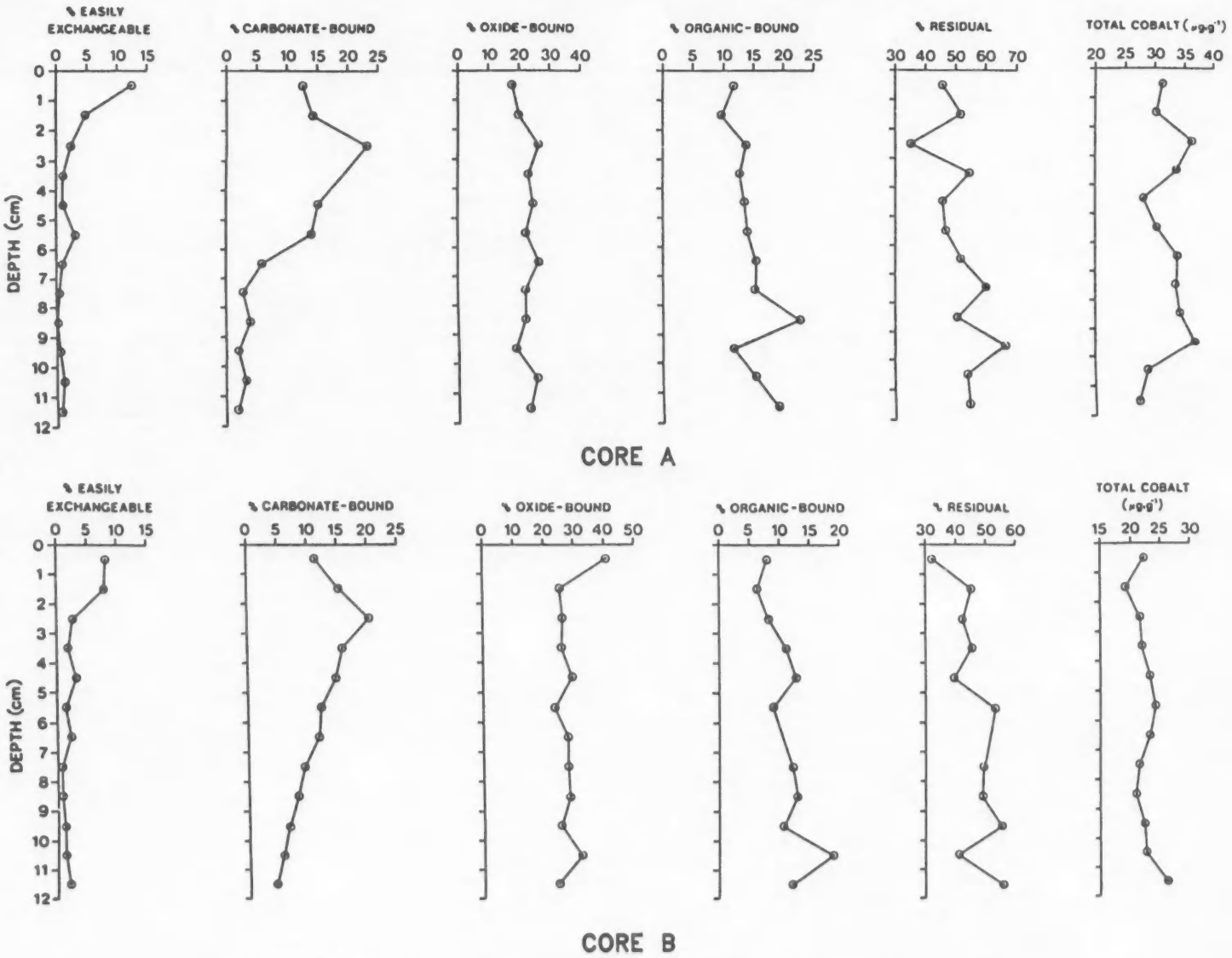

FIG. 3. Profiles of the percent of the stable Co content in five chemical fractions of the sediments in cores A and B.

^{60}Co profile reveals a sharp subsurface maximum (Fig. 2). Since this maximum is not observed in profiles of stable Co, it is believed to be related to higher rates of radionuclide discharge in the past rather than diagenesis within the sediments. This explanation is supported by the presence of a subsurface maximum in ^{137}Cs activity at the same depth (Fig. 2). As expected, ^{60}Co activity in the interstitial water was too low to measure.

Cobalt Fractionation in Sediment Cores

Chemical fractionation of stable Co in the downstream sediments shows that about 50% of the metal is in the residual category (Fig. 3). The easily exchangeable, carbonate-bound, and oxide-bound fractions show distinct trends with depth. The easily exchangeable Co decreases rapidly with depth in the sediment. The carbonate-bound metal almost doubles in the 2- to 3-cm interval and then decreases gradually deeper in the core. The initial decrease in the oxide-bound fraction in core B probably is anomalous and it is not apparent in core A. Organically bound Co increases slightly with depth in the cores despite the decrease in organic content. This increase may result from the peroxide extraction procedure which can dissolve sulfide-bound Co (Tessier et al. 1979), a fraction likely increasing with depth.

Measurement of ^{60}Co activity in the sediment fractions (Fig. 4) was hindered by the low activity of many fractions. The percentage of ^{60}Co activity found in each extraction varied widely between fractions in different depth intervals. More sensitive analyses are required to determine if this variation is a function of detection limits.

Diagenesis of Co in the Sediments

In the Ottawa River sediments, Co was found mostly in the residual (40–60%) and oxide (20–30%) fractions. This fractionation is consistent with the distribution of Co measured in suspended sediments in rivers (Tessier et al. 1980) and bottom sediments of lakes and estuaries (Nriagu 1980; Salomons and Forstner 1985). The distribution of Co changed over the length of the sediment cores. The amount of Co in the easily exchangeable and carbonate-bound fractions decreased with depth, while the Co concentrations in the organic and residual fractions exhibited the opposite trend.

These changes in partitioning with depth may be due to a historical change in the type of input of Co or may result from diagenesis within the sediment cores as geochemical conditions change. Three lines of evidence suggest that the second hypothesis is correct. First, the concentration of stable Co does not change significantly over the length of the cores. This consistency suggests that inputs have been constant over time or that inputs of Co have been proportional to bulk sediment deposition. Second, in laboratory experiments the partitioning of ^{60}Co added to the sediments changed slowly from a majority in the porewater and exchangeable fractions to a majority in the organic and oxide-bound fractions during the 133-d experiments. Finally, the changing distribution of Co in the cores is consistent with changes observed in the partitioning of other trace metals including Pb (Evans and Parliament 1984) and As and Ni (R. J. Cornett and L. Chant, unpubl. data).

These patterns suggest that a general geochemical process controls the diagenesis of trace metals. These results are consistent with the hypotheses of Campbell and Tessier (1984) that the measured changes in Co partitioning result from the decrease in E_h values down the core. Decreasing E_h will result in the dissolution of $FeCO_3$ and formation of FeS_2. Since Co, Ni, and Pb will sorb onto or be coprecipitated with sulfides (Rapin et al. 1986), this hypothesis provides a simple, general, geochemical mechanism that could induce the changes observed in this and other studies.

Partitioning in Radiolabelled Sediments

^{60}Co added to Ottawa River sediments collected upstream of the CRNL effluent is removed quickly from the interstitial water into the easily exchangeable, carbonate-bound, and Fe/Mn

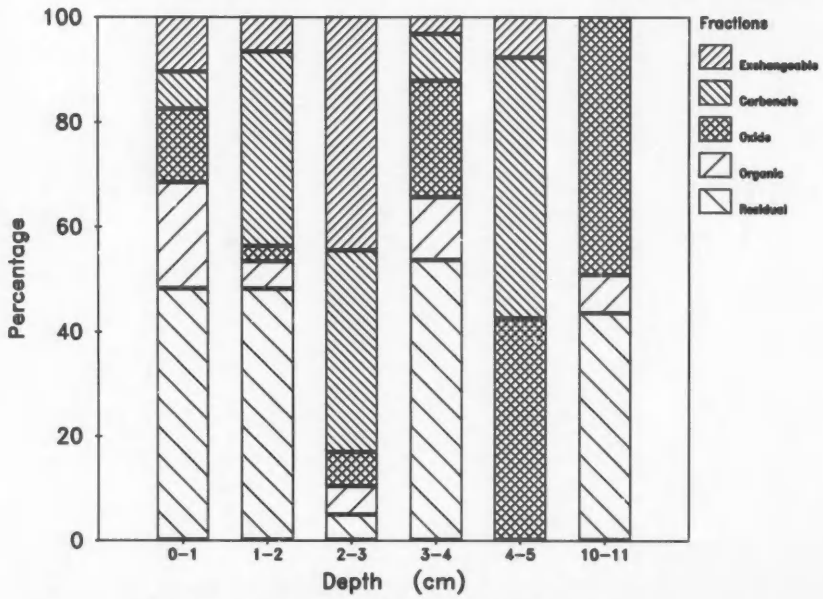

FIG. 4. Fractionation of ^{60}Co in several sections from core B.

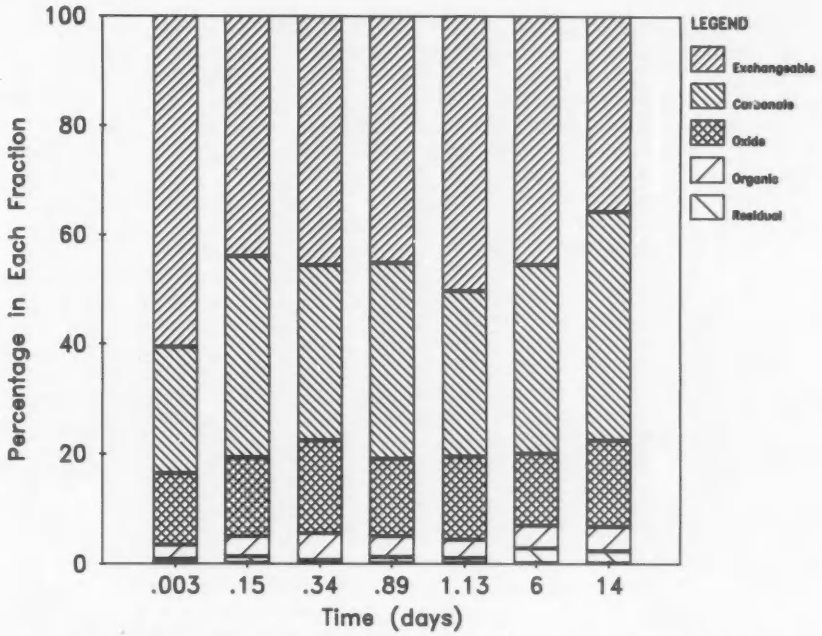

FIG. 5. Fractionation of ^{60}Co added to sediments as a function of time from addition.

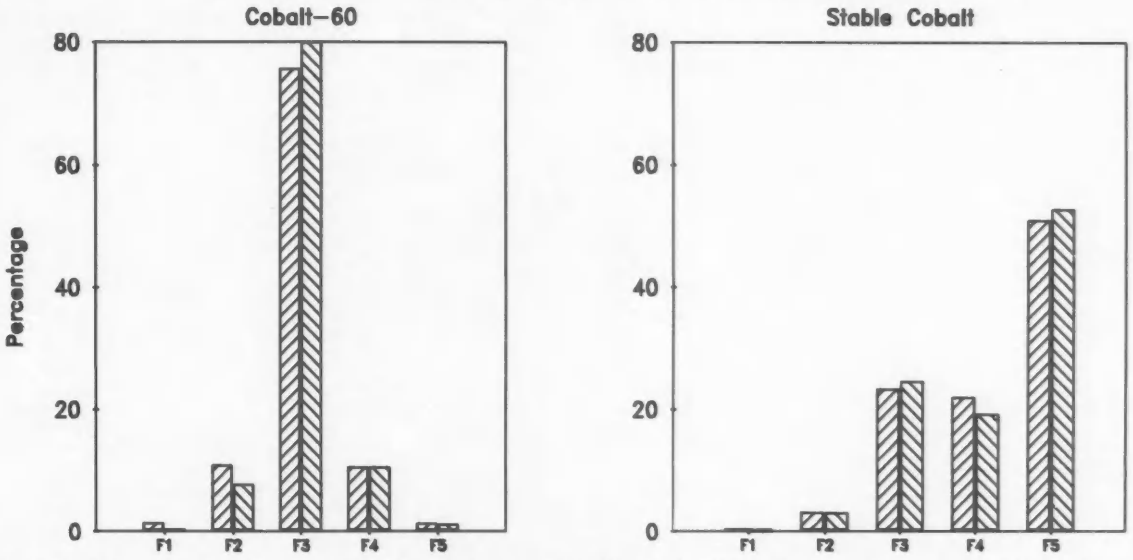

FIG. 6. Replicate analyses of ^{60}Co and stable Co fractionation in spikes sediments after 133 d. (F1 = easily exchangeable, F2 = carbonate-bound, F3 = oxide-bound, F4 = organic-bound, F5 = residual).

oxide-bound fractions (Fig. 5). Minutes after addition, less than 2% of the ^{60}Co remains in the porewater. The ^{60}Co incorporated initially into the easily exchangeable fraction dropped from 164 to 99 $Bq \cdot g^{-1}$ during the first 8 h of the experiment. Much of this loss in ^{60}Co activity from the easily exchangeable fraction is found in the Fe/Mn oxide-bound fraction. Initially, the organic and residual fractions contain very little ^{60}Co. After 14 d, a significant decrease in the easily exchangeable fraction occurred, while amounts in the organic and residual fractions increased.

Sediments collected upstream of the plant, to which ^{60}Co was added, were left to equilibrate for 133 d, after which both ^{60}Co and stable Co were sequentially extracted (Fig. 6). After 133 d, more than 70% of the added isotope was found in the oxide fraction, with virtually no ^{60}Co in either the easily exchangeable or residual fraction. The stable Co, however, did not show a similar distribution (Fig. 6) but rather reflected the partitioning found in the river sediments when collected. These data suggest that Co fractionation shifts towards those compartments that are more difficult to extract.

Co partitioning appears to be related to sediment E_h. As the experiments progressed, it is likely that the sediments became more anoxic. As suggested earlier, this decrease in E_h could result in a shift towards oxide and residual fractions (Campbell and Tessier 1984). Evidence for this mechanism was seen also in an experiment in which river sediments were collected, stirred (and thus reoxidized), and then incubated with and without tubificids (Fig. 7) for 110 d. There is a redistribution of stable Co from the exchangeable and carbonate fractions into the oxide fraction through time similar to that seen in the radiolabelled sediments (Fig. 6). The percentage change in stable Co partitioning is smaller than that of ^{60}Co measured in earlier experiments, since the sizes of the pools at the start of the experiment were quite different. The presence or absence of animals had no effect on the partitioning (Fig. 7). As well, there was no measurable change in Co concentration in either the organic or residual fractions. There appears to be two phases to the kinetics of diagenesis. The first phase is a rapid loss of Co from the interstitial water to the easily exchangeable and carbonate-bound fractions over a few hours. The second phase is a much slower movement of Co into the oxide fraction and to a lesser extent the organic-bound and residual fractions. This phase appears to take about 100 d to reach equilibrium. There may be a much slower movement into the residual fraction but our data do not provide information on this possibility.

Uptake and Bioavailability of Co and ^{60}Co

The Co content of benthic fauna collected from the Ottawa River was less than that of the bulk sediments when both measures were expressed per gram dry weight. For several types of benthos (chironomids, oligochaetes, and amphipods), Co concentrations ranged from 1.4 to 4.4 μg·g dry weight^{-1} (mean = 2.9 ± 1.1) compared with the concentration in the bulk sediments of 20–30 μg·g dry weight^{-1} (Fig. 2). In Round Lake, a small hardwater lake in southern Ontario, the Co content of these macrofaunal families was determined to be in the same range (mean = 2.0 ± 0.4 μg·g^{-1}). These Co concentrations fall within the range observed in other deposit-feeders living in sediments with similar Co concentrations (Bryan and Hummerstone 1977, 1978).

The amount of Co accumulated in organisms incubated with river sediments in the laboratory was small also. Faunal concentrations approached only about one third of those in organisms native to the sediments. The total Co concentration of *Tubificid* sp. increased during the first 50 d of exposure from a mean of 0.47 (t = 0) to 0.90 μg·g dry weight^{-1} with little change in concentration of the animals between 50 and 110 d. High standard deviations (15–100%, n = 10) in replicate samples indicate that uptake of Co by *Tubifex* is quite variable. There was no detectable uptake of ^{60}Co from these sediments.

Because uptake of ^{60}Co from Ottawa River sediments was not detectable, *Tubifex* worms were cultured in sediments spiked with higher ^{60}Co activity. ^{60}Co activity in *Tubifex* in these experiments was measured easily. For unaccountable reasons, the organisms in these experiments assimilated six times more stable Co than *Tubifex* cultured for 110 d and reported above. The mean concentration after 110 d was 6.0 ± 0.8 μg Co·g dry weight^{-1}. The mean specific activity of ^{60}Co in the organisms was 1400 ± 200 Bq·μg Co^{-1} and much less variable than the stable Co concentration.

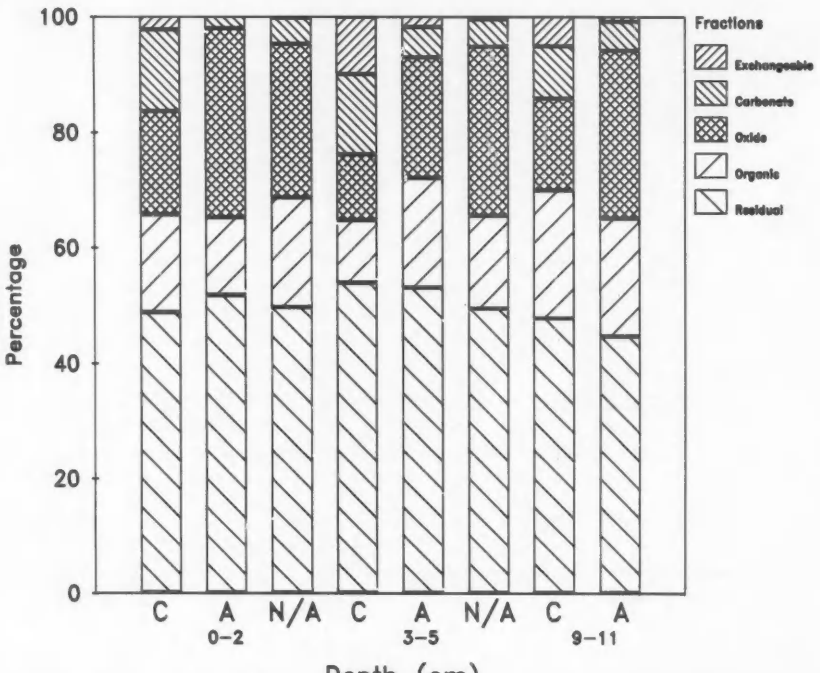

FIG. 7. Fractionation of stable Co in experimental sediments with and without the addition of *Tubifex*. (C = control at start of experiment, A = after 110 d with animals added, N/A = after 110 d with no animals added).

TABLE 1. Specific activity of ^{60}Co in sediments in replicate experiments 133 d after spiking with 5 μCi.

Fraction	Specific activity of ^{60}Co	
Easily exchangeable	1155	1646
Carbonate-bound	1053	905
Oxide-bound	1046	1161
Organic-bound	152	197
Residual	9	9

Chemical partitioning of the sediments at the conclusion of incubation (Fig. 6; Table 1) showed that only the easily exchangeable, carbonate-bound, and oxide-bound fractions had high enough specific activities to account for the high specific activities in the organisms. The organically bound and residual Co fractions had Co specific activities one and two orders of magnitude less, respectively. The difference in specific activities eliminates these two fractions as possible sources of Co to the worms, in spite of the high percentage of the total Co in these fractions (>72%). The other potential source of Co to these organisms is the interstitial water. The specific activity of this compartment (approximately 2000 $Bq \cdot \mu g^{-1}$) is higher than that found in the organisms. However, over the incubation period, the organisms accumulated a mass equivalent to 133 and 100% of the stable Co and ^{60}Co, respectively, which was present in the interstitial water. Thus, it is unlikely that the interstitial water is the major source of Co, but on the basis of our experiments, it is not possible to eliminate it entirely.

An alternative means of determining the significance of the Co in the exchangeable, carbonate, and oxide fractions to organism accumulation is an analysis of concentration factors (CF = ratio of concentration in organisms to concentration in the sediment). The average concentration factors for stable Co and ^{60}Co were 0.3 and 1.4, respectively, suggesting that the ^{60}Co is more readily available to the worms than the stable Co. However, if we express the CF for stable Co using the sum of the amounts found in the first three fractions only (i.e. those fractions with the specific activity closest to that found in the organisms), the CF is 1.2. This CF is identical to the calculated CF for radioactive Co added to the sediments. Thus, only that stable Co found in the first three sediment fractions is bioavailable although the majority is found in the organic and residual fractions (Fig. 6).

Our results are consistent with several studies showing that the biological availability of stable trace metals is predicted poorly by the total sediment concentration (Bryan and Hummerstone 1977; Skoch and Sikes 1973). Chemical fractionation of sediments by operational procedures may yield better predictions of body burdens of trace metals, such as Co, in benthic fauna (Luoma 1983). For example, Luoma and Bryan (1978) related the Pb concentration in a deposit-feeding bivalve, *Scrobicularia*, to the ratio Pb/Fe in a 0.1 *N* HCl extract of the sediment, an extract supposed to resemble the gut environment. Luoma and Jenne (1976a, 1976b) found good correlations between animal uptake of ^{65}Zn and ^{109}Cd by the bivalve *Macoma balthica* and readily exchangeable ^{65}Zn and ethanol-extracted ^{109}Cd in the sediments. Tessier et al. (1984) related the uptake of Cu by a freshwater mollusc, *Elliptio complanata*, to the sum of easily exchangeable, carbonate-bound, and oxide-bound Cu divided by oxide-bound Fe in the sediments. Campbell et al. (1985) related Cu in the rhizomes of the yellow pond lily *Nuphar* to easily exchangeable and carbonate-bound Cu in the sediments, again divided by exchangeable or oxide-bound Fe.

From these studies, it is apparent that the available portion of trace metals varies among systems. In the absence of a predictive theory to explain this variation, our study indicates that a comparison of specific activities in organisms with those of different sediment fractions can be used to determine which chemical fractions are the source of the metal and its radioisotope analogue. We encourage additional studies of the specific activity approach in other environments.

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Adaptive Variation in Rheotactic and Agonistic Behavior in Newly Emerged Fry of Chinook Salmon, *Oncorhynchus tshawytscha*, from Ocean- and Stream-Type Populations

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Taylor, E. B. 1988. Adaptive variation in rheotactic and agonistic behavior in newly emerged fry of chinook salmon, *Oncorhynchus tshawytscha*, from ocean- and stream-type populations. Can. J. Fish. Aquat. Sci. 45: 237–243.

Agonistic and rheotactic behavior and body morphology were compared in recently emerged, laboratory-reared chinook salmon (*Oncorhynchus tshawytscha*) from two "stream-type" and two "ocean-type" populations. Newly emerged chinook fry from the stream-type populations (Slim Creek and the Eagle River) were more aggressive than fry from the ocean-type populations (the Nanaimo and Harrison rivers). Slim Creek fry were consistently the most aggressive. There was no clear distinction in rheotactic behavior between stream- and ocean-type chinook; Harrison River, ocean-type chinook fry had the strongest downstream movement in "dark" current response tests, but fry from the other three populations had similar movement scores in both light and dark tests. Fry from the four populations were morphologically distinct; however, there was no clear separation in body morphology or coloration based on life history type. These differences exhibited in laboratory-reared fry indicate that they are, at least in part, inherited. I conclude that a fundamental genetic difference in agonistic behavior exists between stream- and ocean-type chinook juveniles. A genetic dichotomy between stream- and ocean-type chinook in rheotactic behavior and morphology, however, may be overidden by population-specific local adaptations, independent of life history type.

On a comparé le comportement agonistique et rhéotaxique et la morphologie du corps d'alevins à vésicule résorbée de saumon quinnat (*Oncorhynchus tshawytscha*) appartenant à deux populations « dulçaquicoles » et deux populations « marines » élevées en laboratoire. Les alevins à vésicule résorbée provenant des populations dulçaquicoles (ruisseau Slim et rivière Eagle) étaient plus agressifs que les alevins de populations marines (rivières Nanaimo et Harrison). Ceux venant du ruisseau Slim étaient uniformément plus agressifs. Il n'y avait toutefois pas de différence évidente du comportement rhéotaxique chez les quinnats dulçaquicoles et marins; c'est chez les quinnats marins de la rivière Harrison que l'on a observé les plus grands déplacements vers l'aval au cours d'expériences de réaction au courant à la noirceur mais on a observé des résultats semblables chez les alevins des trois autres populations soumis à la lumière et à la noirceur. Les alevins des quatre populations étaient différents au niveau morphologique; toutefois, il n'y avait pas de différence évidente de la morphologie ou de la coloration du corps découlant du cycle vital. Les différences révélées chez des alevins élevés en laboratoire révèlent qu'elles sont au moins en partie héritées. L'auteur formule la conclusion qu'il existe une différence génétique fondamentale du comportement agonistique entre les juvéniles dulçaquicoles et marins. Des adaptations locales particulières à chaque population, indépendantes du type de cycle vital, peuvent toutefois l'emporter sur la dichotomie génétique du comportement rhéotaxique et de la morphologie chez les quinnats dulçaquicoles et marins.

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The chinook salmon (*Oncorhynchus tshawytscha*) exhibits a wide range in the length of time juveniles spend rearing in freshwater before migrating seaward as smolts (Reimers 1973; Healey 1983; Carl and Healey 1984). Juveniles that migrate to sea as newly emerged fry or after a few months in freshwater are called "ocean-type" whereas those that rear in freshwater for extended periods and migrate to sea as age 1+ smolts are called "stream-type."

Apart from differences in scale growth pattern (Gilbert 1913; Reimers 1973), studies by Healey (1983) and Carl and Healey (1984) have documented distributional, electrophoretic, and morphological differences between stream- and ocean-type chinook within and among populations. Taylor and Larkin (1986) reported differences in morphology and rheotactic and agonistic behaviors between stream-type and ocean-type wild chinook fry from two tributaries of the Fraser River (Slim Creek and the Harrison River). Newly emerged, stream-type fry from Slim Creek were less likely to move downstream in a current channel, were more aggressive, had more parr markings, and had larger body parts and larger and more brightly colored median fins than fry from the Harrison River, an ocean-type population. These behavioral and morphological differences are

similar to those among juveniles of *Oncorhynchus* (e.g. pink (*O. gorbuscha*) and coho (*O. kisutch*) salmon) which exhibit a similar range of freshwater life history patterns to that present among chinook life history types. Coho salmon rear in freshwater for a year or more are territorial, have relatively deep bodies with distinct parr markings, and have large, brightly colored median fins. In contrast, pink salmon migrate seaward as newly emerged fry, are schooling, nonterritorial, relatively slim-bodied, lack parr marks, and have smaller, transparent median fins (Hoar 1951, 1976; Chapman 1962).

If natural selection is responsible for the divergent life history patterns of chinook salmon fry, then this variability must have a genetic basis. The goal of the present study was to test whether the behavioral and morphological differences between stream-type and ocean-type chinook fry are inherited. Environmental variation between the populations was eliminated by studying chinook fry which had been incubated and reared in the laboratory under identical conditions.

Materials and Methods

Study Populations

Two of the chinook populations studied were Slim Creek (stream-type) and the Harrison River (ocean-type), two tributaries of the Fraser River as described by Taylor and Larkin (1986). An additional stream-type population came from the Eagle River, another tributary of the Fraser River which enters Shuswap Lake near Sicamous, B.C. From scale analysis of adults, Eagle River chinook are largely (>95%) stream-type and juveniles overwinter in freshwater (Shepherd et al. 1986b). An additional ocean-type population came from the Nanaimo River, on southeastern Vancouver Island near Nanaimo, B.C. I studied the "fall run" subpopulation which spawns in the lower Nanaimo River (see fig. 1 of Carl and Healey 1984). This lower river subpopulation produces fry which are predominantly (>98%) ocean-type, moving downstream to the Nanaimo estuary soon after emergence (Healey 1980; Carl and Healey 1984).

Gamete Collection and Incubation

Eggs and milt were collected from ripe, wild chinook salmon during September (Slim Creek and Eagle River), October (Nanaimo River), and November (Harrison River) of 1985. Slim Creek adults were collected on the spawning grounds by electroshocking, while adults from the Eagle, Harrison, and Nanaimo rivers were collected at fences or by seining in conjunction with enhancement operations on these rivers. Eggs were fertilized in the field by mixing eggs and milt from a small number of females and males (Slim Cr., 4 females × 7 males; Eagle R., 6 females × 4 males; Nanaimo R., 3 females × 4 males; Harrison R., 3 females × 2 males). Water-hardened zygotes were transported to the laboratory in 4-L glass jars filled with water. During transport, water temperature was maintained as close as possible to 5°C by placing the glass jars in coolers filled with crushed ice. Elasped time from fertilization to placement in the incubator ranged from 2 h (Harrison River) to 24 h (Slim Creek).

The incubation apparatus consisted of a large (1.8 × 0.6 × 0.9 m) cooling unit supplied with dechlorinated water at about 2 L·min^{-1}. Within the cooling unit, zygotes were incubated in "corner-type" aquarium filters with about 3–4 cm of pea gravel provided as substrate. Each filter had its own air supply which generated a flow of water over the incubating zygotes and larvae. The incubation temperature was set at 5°C; actual incubation temperature over the 7-mo incubation period was 4.5 ± 0.86°C.

TABLE 1. Mean (± SE standard lengths (SL, mm) and weights (Wt, g) of laboratory-reared chinook fry from the four study populations before and at the end of the aquaria tests. N = 25 for each population.

	Slim Cr.		Eagle R.		Nanaimo R.		Harrison R.	
	SL	Wt	SL	Wt	SL	Wt	SL	Wt
Start	31.9 (0.16)	0.39 (0.01)	30.3 (0.36)	0.31 (0.01)	34.9 (0.46)	0.46 (0.02)	34.1 (0.25)	0.44 (0.01)
Finish	32.6 (0.12)	0.42 (0.01)	31.9 (0.23)	0.38 (0.02)	36.5 (0.29)	0.48 (0.01)	35.6 (0.24)	0.49 (0.02)

Upon emergence, fry were placed in 94-L fiberglass rearing troughs where they were left for 24 h. The water flow through the troughs was maintained at 4 L·min^{-1} and fry were fed Oregon Moist Pellet (OMP) food thrice daily. Daily light schedule followed the natural photoperiod and was provided by a single 40-W fluorescent bulb per trough. A single 25-W light bulb controlled by a rheostat-timer was suspended over each trough to provide a "dawn–dusk" period each day.

Chinook Fry Behavior

I studied rheotactic behavior in the newly emerged fry during current response tests run in two artificial, oval-shaped stream channels (3.8-m stretched length). The current channels and test protocol were as described by Taylor and Larkin (1986). Both current channels were subdivided into 22 compartments ranked from −10 (most downstream) through 0 (central compartment) to +10 (most upstream). Each trial consisted of assessing the movements of 20 chinook fry through the channel compartments during successive "light" and "dark" tests. Each test was 2.5 h in duration with 0.5 h between successive tests. For each test a movement score was calculated by summing the products of the number of fish in a compartment multiplied by the rank of that compartment and dividing by 20, the number of fish in each test. To provide a net movement score the downstream score was subtracted from the upstream score; a constant of 10 was added to eliminate negative scores. Twenty trials, each consisting of 20 "dark" and 20 "light" tests, were run for each population. Water temperature was maintained between 10 and 10.5°C for all tests and water velocity through the channel compartment openings averaged about 10 cm·s^{-1}. During the period of spring fry migration, 10°C is within the range of temperatures in the four study streams and was the temperature best controlled in the current channels. Data were analysed by two-factor analysis of variance with illumination (light, dark) and population as the main effects.

To study agonistic behavior in fry from the four populations, I recorded the number and duration of agonistic behaviors observed during individual-fry, aquaria tests (10°C) and during multiple-fry, stream tank tests (11°C). In all cases, methods and test conditions were identical to those described by Taylor and Larkin (1986). In the single-fry, aquaria tests, fry were observed for 3-min periods for their reactions to their mirror images (mirror-image-stimulation (MIS, Gallup 1968)). Tests continued for seven consecutive days on 25 fry per population. I used an OS-3 event recorder to score "null," "swim-against-mirror," "lateral display," and "wigwag" behaviors (see Taylor and Larkin (1986) for definitions. Fry were weighed and meas-

TABLE 2. Mean (± SE) standard lengths (mm) of newly emerged chinook and coho salmon fry used in the interspecific stream tank tests. $N = 8$ all cases.

	Stream tank section	
	Section 1	Section 2
Slim Creek chinook fry	32.4 (0.21)	32.5 (0.16)
Coho fry	32.4 (0.26)	32.2 (0.35)
Eagle River chinook fry	33.0 (0.35)	32.5 (0.33)
Coho fry	31.3 (0.46)	31.8 (0.39)
Nanaimo River chinook fry	35.8 (0.31)	35.6 (0.49)
Coho fry	33.8 (0.36)	33.7 (0.31)
Harrison River chinook fry	36.2 (0.38)	35.8 (0.21)
Coho fry	34.4 (0.44)	34.6 (0.41)

ured (standard length) before and after each 7-d test (Table 1). A power failure prevented collection of behavioral data on the seventh day for Eagle River chinook.

In the stream tanks tests, I observed chinook fry during single-species and double-species (with coho salmon) tests. Sixteen newly emerged chinook fry were observed by scan sampling in each of two replicate stream tank sections (see Taylor and Larkin (1986) for description) for 10 min three times per day for five consecutive days. The behaviors recorded were "null," "approach," "lateral display," "wigwag," "charge," and "nip" (see Taylor and Larkin (1986) for definitions). Fry were fed after each observation period and there was at least 2 h between consecutive observations.

Stream tank tests were repeated for each population using eight chinook fry from the test population and eight coho fry (Table 2). Since coho tend to dominate chinook fry in contrived situations (Stein et al. 1972; Taylor and Larkin 1986), these interspecific tests provide another measure of the relative agressiveness of chinook fry from each population. The tests were identical to the single-species tests and lasted for five consecutive days. The coho fry were progeny from crosses of adults spawning in Black Creek, east-central Vancouver Island and were also incubated and reared in the laboratory. Data in the MIS aquaria tests and the stream tank tests were analysed by two-factor repeated measures ANOVA with population and observation day as the main effects.

Chinook Fry Morphology

Morphological analysis on preserved (10% buffered formalin) newly emerged chinook fry consisted of body measurements and counts (left side only). All measurements were as in Taylor and Larkin (1986) and followed Hubbs and Lagler (1967). All measures except standard length were made under a dissecting microscope fitted with a micrometer eyepiece. Standard length was measured with Vernier calipers. A general evaluation of coloration of the body and fins of fry was made on anaesthetised specimens after the stream tank tests. To minimize size-related differences in body shape, body measurements were adjusted to the overall sample mean standard length. The adjustment procedure used was covariance analysis on $\log_{10}$-transformed data, and t-tests assessed differences in the adjusted means among the study populations.

Results

Rheotactic Behavior

In the current channels, the laboratory-reared chinook fry from all populations responded by "holding" or swimming slightly upstream during light tests while moving downstream during dark tests (light versus dark factor, $p < 0.0001$). In addition to moving in opposite directions in light and dark tests, fry movement tended to be more pronounced during dark tests, with all populations showing significant downstream movement (t-tests for $\mu = 10$, all $p < 0.001$), while during light tests, all fry except those from the Harrison River showed slight, but significant, upstream movement ($p < 0.05$) (Table 3). Ocean-type fry from the Harrison River had a significantly lower overall mean net movement score ($p < 0.0001$) than fry from the three other populations. This overall difference, however, was largely due to significantly greater downstream movement by Harrison River fry during dark tests, since no significant differences in net movement scores were evident among the four populations during light tests (population × illumination factor, $p < 0.01$). In order of increasing downstream movement in the dark the populations ranked Nanaimo, Slim, Eagle, and Harrison whereas in order of increasing upstream movement in the light the populations ranked Harrison, Eagle, Slim, and Nanaimo.

TABLE 3. Mean net movement scores (± SE) of laboratory-reared, newly emerged chinook fry from the four study populations. Underlining indicates those mean scores which are not significantly different ($p > 0.05$). A score of 0 = maximum downstream movement, 10 = no net movement upstream or downstream, and 20 = maximum upstream movement. $N = 20$ in all cases.

	Slim Cr.	Eagle R.	Nanaimo R.	Harrison R.
Light tests	11.74 (0.43)	10.90 (0.47)	12.28 (0.41)	10.44 (0.44)
Dark tests	6.37 (0.44)	6.14 (0.44)	7.48 (0.42)	3.02 (0.43)
Overall	8.99 (0.31)	8.51 (0.35)	9.98 (0.38)	6.64 (0.31)

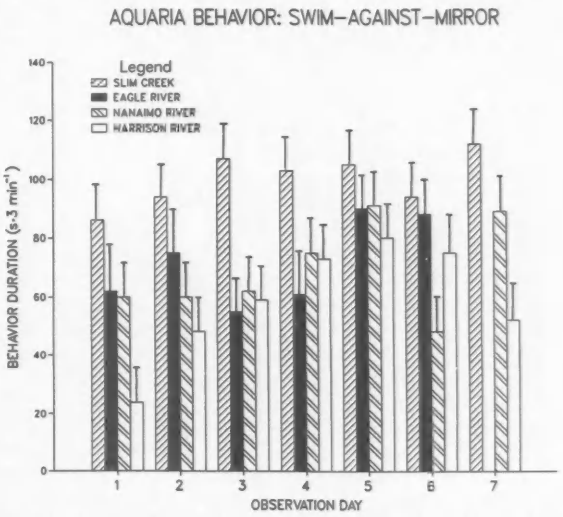

FIG. 1. Durations (mean ± SE) of swim-against-mirror behavior for newly emerged chinook salmon fry from Slim Creek, Eagle River (stream-type), Nanaimo River, and Harrison River (ocean-type). $N = 25$ fry per population per day.

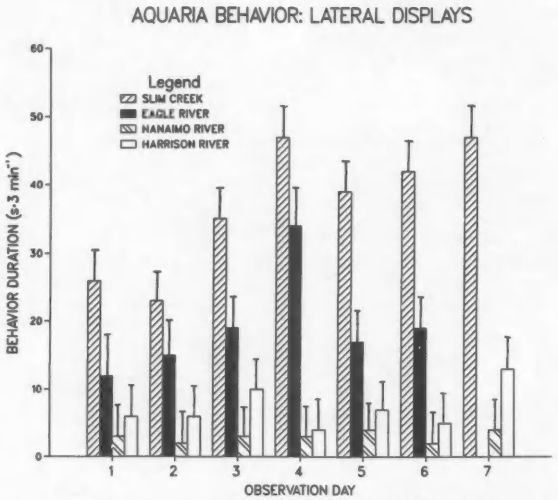

FIG. 2. Durations (mean ± SE) of lateral display behaviors for newly emerged chinook salmon fry from Slim Creek, Eagle River (stream-type), Nanaimo River, and Harrison River (ocean-type). $N = 25$ fry per population per day.

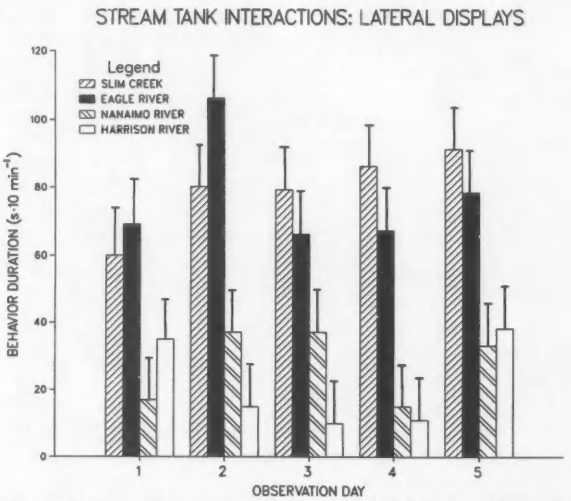

FIG. 3. Durations (mean ± SE) of lateral display behaviors for newly emerged chinook salmon fry from Slim Creek, Eagle River (stream-type), Nanaimo River, and Harrison River (ocean-type) during intraspecific stream tank tests. N = six 10-min observation periods for 16 fry per population per day.

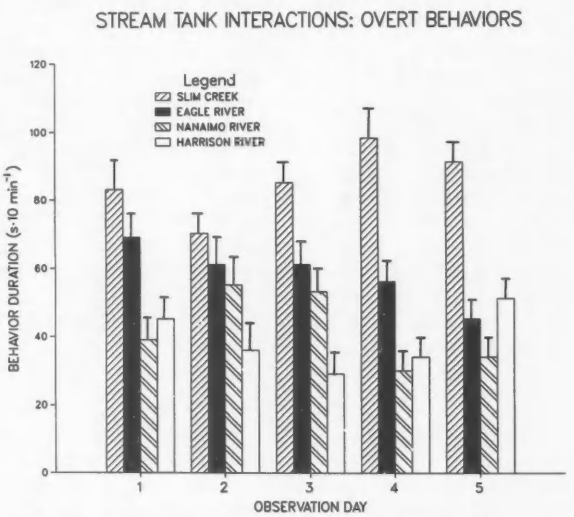

FIG. 4. Durations (mean ± SE) of overt behaviors for newly emerged chinook salmon fry from Slim Creek, Eagle River (stream-type), Nanaimo River, and Harrison River (ocean-type) during intraspecific stream tank tests. N = six 10-min observation periods for 16 fry per population per day.

Agonistic Behavior

In the MIS tests, most fry adjusted quickly to the presence of the mirror in their aquaria. Fry typically reacted by orienting towards their mirror image and then generally approaching the mirror followed by bouts of "swim-against-mirror" and "lateral displays." Both the time it took fry to show some overt reaction to their mirror image and to approach it decreased over the 7-d tests. Fry were occasionally observed to "charge" and even "nip" their mirror images, but these behaviors were observed so infrequently that they were not included in the analysis. The laboratory-reared chinook fry from all populations responded similarly by becoming more aggressive over the 7-d test period (Fig. 1, day factor $p < 0.03$, population × day factor $p > 0.5$). The populations ranked Slim > Eagle > Nanaimo > Harrison in terms of mean time spent in "swim-against-mirror" over 7 d; however, there was only one significant difference: Slim Creek fry were more aggressive than the fry from the three other populations ($p < 0.001$).

For "lateral display" behaviors, which included "horizontal," "head-up," and "head-down" wigwags (North 1979), laboratory-reared, stream-type chinook fry from both the Eagle River and Slim Creek spent significantly more time performing lateral displays than ocean-type fry from the Nanaimo and Harrison rivers (Fig. 2, $p < 0.001$). Slim Creek fry, and to a lesser extent Eagle River fry, tended to spend more time performing "lateral display" behaviors as the tests proceeded whereas Nanaimo and Harrison fry had consistently low display durations (day factor $p < 0.02$). The interaction between population and day effects, however, was not significant ($p > 0.1$). Overall, Slim Creek fry were again the most aggressive fry ($p < 0.001$) with greater "lateral display" durations than the fry from the three other populations (Fig. 2).

Stream Tank Tests: Intraspecific Tests

During the stream tank tests the stream-type chinook fry were significantly more aggressive than their ocean-type counterparts (Fig. 3, 4). The laboratory-reared fry from Slim Creek and the Eagle River spent more time performing both overt ("approach," "charge," and "nip") and lateral display behaviors than did the fry from the Nanaimo or Harrison rivers (both $p < 0.001$). Fry from Slim Creek again proved to be the most aggressive fry, since they spent more time performing overt behaviors ($p < 0.001$) than all other fry whereas fry from both ocean-type populations spent similar durations to each other performing agonistic behaviors (both $p > 0.05$). The four populations were most similar in overt behavior durations during observation days 2 and 3; the greatest differences were observed

TABLE 4. Mean (± SE) frequency of agonistic behaviors observed per 10-min observation period for laboratory-reared, newly emerged chinook fry from the four study populations during intraspecific stream tank tests. Underlining indicates means which are not significantly different ($p > 0.05$). $N = 30$ in all cases.

Behavior	Slim Cr.	Eagle R.	Nanaimo R.	Harrison R.
Lateral displays	27.0 (1.9)	12.0 (1.9)	11.6 (1.9)	9.2 (1.9)
Wigwags	21.3 (2.1)	33.0 (2.2)	5.5 (2.2)	4.6 (2.1)
Total displays	48.2 (3.3)	45.1 (3.5)	17.1 (3.3)	13.8 (3.3)
Approach	51.5 (2.5)	44.7 (2.6)	25.8 (2.5)	22.8 (2.5)
Charge	17.1 (1.2)	6.1 (1.1)	11.1 (1.2)	11.2 (1.2)
Nip	26.2 (2.1)	10.1 (2.2)	22.5 (2.1)	19.2 (2.1)
Total overt	95.1 (5.0)	56.1 (5.2)	59.4 (5.0)	53.1 (5.0)

on observation days 1, 4, and 5 (Fig. 4, population × day, $p < 0.05$).

As might be expected from the behavior durations, laboratory-reared, stream-type chinook fry from Slim Creek and the Eagle River performed significantly more lateral display behaviors than ocean-type fry (Table 4), with Slim Creek fry performing the most "lateral displays" and Eagle River fry performing the most "wigwag" displays. Fry from the Nanaimo and Harrison rivers did not differ from each other in frequency of lateral display behaviors (Table 4). For overt behaviors, Slim Creek fry were consistently the most aggressive performing more "approaches" and "nips" than fry from the other populations, while Eagle River fry also performed more "approaches" than ocean-type fry. In contrast, while Nanaimo and Harrison River fry "charged" less frequently than Slim Creek fry, they both "charged" more frequently than stream-type, Eagle River fry. In addition, Nanaimo River fry "nipped" at the same rate as Slim Creek fry, but significantly more frequently than Eagle River chinook fry.

Stream Tank Behavior: Interspecific Tests

In the stream tank tests where coho and chinook fry were studied together, coho tended to dominate chinook (Fig. 5, $p < 0.0001$). A net aggression score was calculated for each observation period by taking the ratio of the sum of all aggressive behavior durations of chinook directed towards coho to the sum of all aggressive behavior durations of coho directed towards chinook. As values of this ratio approach 0, increasing domination of coho is indicated whereas values approaching 1 indicate increasingly equal interaction between the species. Since all mean values are <1, clear domination of chinook by coho is indicated (Fig. 5). The domination by coho was especially evident as the tests proceeded, since mean values tended to decrease over the 5-d tests for all populations except during the Harrison River tests. A few of the trials involving Eagle River chinook and Black Creek coho resulted in net domination by chinook. Slim Creek and Eagle River, stream-type fry, however, tended to be more aggressive towards coho than were the ocean-type chinook fry (Fig. 5, $p < 0.005$).

Morphology and Coloration

Morphological analysis suggested no stream/ocean-type dichotomy with respect to body form or coloration among the

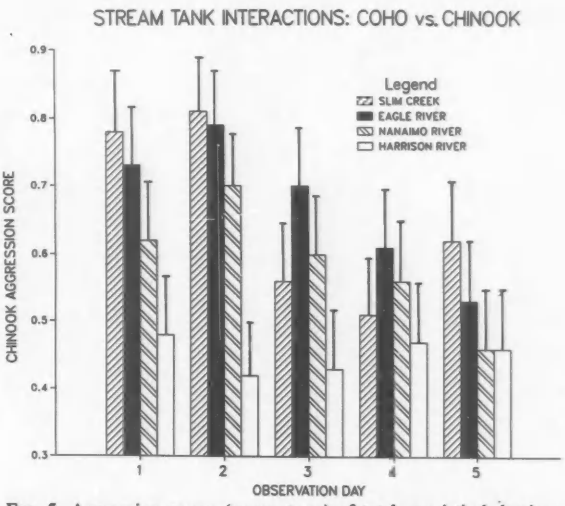

FIG. 5. Aggression scores (mean ± SE) of total agonistic behaviors of newly emerged chinook fry from Slim Creek, Eagle River (stream-type), Nanaimo River, and Harrison River (ocean-type) in stream tank tests with coho salmon fry. See text for explanation of score calculation. N = six 10-min observation periods for eight chinook and eight coho fry per day.

laboratory reared chinook fry from the four study populations (Table 5). While chinook from Slim Creek and the Eagle River had the deepest bodies and heads, it was Slim Creek fry and ocean-type, Nanaimo River fry which had the largest median fins. In fact, while Slim Creek fry had consistently the largest fins, in all but one fin measure (af1), Eagle River, stream-type fry had the smallest fins. The populations also differed in the number of parr marks with fry from the Nanaimo and Eagle rivers having the most major and total parr marks (Table 5).

All fry possessed black and white leading edges on the dorsal fin and white tips on their anal fins. The area of greatest distinction with respect to fin coloration among the four populations was in the degree of orange pigment in the median and paired fins. Slim Creek fry were the most elaborately colored fry with prominent orange coloration on all median and paired fins. Nanaimo fry were also well colored, but they lacked the bright orange–black color of the Slim Creek fry caudal fins and orange coloration in the paired fins. Stream-type fry from the Eagle River tended to be lighter in color than fry from either Slim Creek or the Nanaimo River. While Eagle River chinook fry had orange pigment in their median fins, it was not universal, especially in the caudal fin. In addition, many Eagle fry lacked the orange mandible stripe characteristic of the Slim Creek and Nanaimo River fry. The Harrison River, ocean-type fry were the least brightly colored. Harrison fry lacked any trace of orange pigment in their fins, but had some faint yellow color in the caudal fin. While Slim and Nanaimo fry were dark brown with a distinct purple sheen to their sides, Eagle River fry were a lighter grey–brown along their sides. Harrison River fry were greyish–green along their sides and became "silvery" upon preservation in formalin.

Discussion

The behavioral and morphological differences between laboratory-reared, stream-type Slim Creek chinook fry and

TABLE 5. Geometric mean (± SE) standard lengths (mm) and adjusted mean body proportions and parr mark counts of newly emerged, laboratory-reared chinook fry from the four study populations. $N = 30$ in all cases and measurements were adjusted to an overall sample mean standard length of 30.6 mm by covariance analysis. The indicated probability is that all adjusted means are the same.

Character	Slim Cr.	Eagle R.	Nanaimo R.	Harrison R.	p
Standard length	30.5 (0.22)	28.6 (0.20)	32.7 (0.29)	31.2 (0.16)	—
Body depth	6.3 (0.06)	6.4 (0.08)	5.7 (0.08)	5.9 (0.06)	<0.001
Head depth[a]	5.8 (0.05)	5.5 (0.06)	5.4 (0.06)	5.4 (0.05)	<0.001
Head length[a]	8.6 (0.05)	8.2 (0.07)	8.5 (0.07)	8.6 (0.05)	<0.001
Dorsal fin height	3.7 (0.07)	2.8 (0.09)	3.1 (0.09)	2.9 (0.07)	<0.001
Dorsal fin length	6.3 (0.07)	5.2 (0.09)	5.7 (0.09)	5.8 (0.07)	<0.001
Anal fin height	3.5 (0.05)	2.8 (0.07)	3.0 (0.07)	2.9 (0.05)	<0.001
Anal fin length	4.8 (0.09)	4.2 (0.12)	4.2 (0.11)	4.4 (0.09)	<0.001
Caudal fin depth[a]	7.7 (0.14)	7.5 (0.18)	7.6 (0.18)	7.5 (0.14)	>0.05
Parr mark length	3.1 (0.11)	2.6 (0.14)	2.5 (0.13)	2.8 (0.10)	<0.005
Parr mark width	1.8 (0.04)	1.4 (0.05)	1.3 (0.05)	1.3 (0.04)	<0.001
No. of major parr marks	9.2 (0.21)	10.6 (0.29)	11.4 (0.28)	10.1 (0.22)	<0.001
No. of total parr marks	16.7 (0.73)	18.4 (0.97)	21.1 (0.93)	15.1 (0.74)	<0.001

[a]Heterogenous standard length – character regression slopes ($p < 0.05$)

ocean-type fry from the Harrison River demonstrated in the present study are similar to the differences between wild-reared fry from these populations (Taylor and Larkin 1986). In both cases, Slim Creek newly emerged fry were more aggressive, less likely to move downstream in the current channels, had larger body parts and median fins, and were more colorful than Harrison River fry. The maintenance of these phenotypic differences in fry reared in the laboratory demonstrates that they are, at least in part, genetic in origin. The inclusion of fry from the Nanaimo and Eagle rivers further demonstrates genetic differentiation in agonistic behavior between stream- and ocean-type chinook fry. Slim Creek fry were, almost without exception, the most aggressive fry whereas Harrison fry tended to be the least aggressive of the four populations. Eagle River and Nanaimo River chinook were intermediate to Slim and Harrison chinook, usually ranking second and third, respectively, in agonistic behavior durations (e.g. Fig. 3). Thus, the stream-type chinook fry (Slim and Eagle) tended to be more aggressive than the ocean-type fry (Nanaimo and Harrison). Unique selective regimes imposed by the different freshwater rearing patterns between stream- and ocean-type chinook juveniles probably account for the higher aggression levels in stream-type chinooks. The elaborate displays often involved in aggressive behavior of juvenile salmonids, and the clear contradictions involved in territorial aggression and seaward migration may have resulted in selection against agonistic behavior in ocean-type populations.

In contrast to the differences in agonistic behavior, no strict stream/ocean-type dichotomy is evident with respect to rheotactic behavior or morphology. The lack of a clear distinction between stream- and ocean-type chinook fry in rheotactic behavior and morphology may result from a tempering of differences between life history types by local conditions unrelated to the length of freshwater residence. For instance, while Slim and Eagle fry moved downstream less readily than Harrison River fry, Nanaimo River, ocean-type fry had the highest net movement scores in both light and dark tests (Table 3). Since Carl and Healey (1984) suggested that the lower Nanaimo River chinook subpopulation is characterized by fry which migrate to the estuary soon after emergence, this result was unexpected. The Nanaimo River estuary is, however, within 5 km of the lower spawning area. In order to reach the estuary soon after emergence, lower Nanaimo chinook fry probably do not require as strong a downstream response as fry from other ocean-type populations such as the Harrison River from which fry must migrate about 100 km to the Fraser River estuary. For Nanaimo River fry a more gradual downstream drift, similar to the behavior of Slim Creek and Eagle River fry, would accomplish this in a short time. Hoar (1976) reported that newly emerged chinook fry require some acclimation time for survival at salinites above 15‰. Since salinities in the Nanaimo River estuary are commonly above 20‰ (Healey 1980), a more gradual movement into the estuary by Nanaimo fry may be necessary to prevent osmoregulatory shock. Healey (1980) also reported that mean weights of chinook fry entering the Nanaimo estuary were about 0.57 g. The fry used in the present study, while completely "buttoned-up," were about 20% lighter (0.30–0.53 g). Perhaps, through a rearing migration, some growth in freshwater must occur before Nanaimo chinook can successfully enter the estuary. Thus, as a consequence of the different migration distances to their respective estuaries, my results suggest that ocean-type chinook from the Nanaimo and Harrison rivers are genetically distinct in their manner of downstream migration as newly emerged fry; Harrison River fry exhibit a more pronounced, directed nocturnal downstream movement.

Furthermore, nocturnal downstream dispersal to freshwater rearing areas is commonly observed in newly emerged fry of various stream-resident salmonids (e.g. Reimers 1973; Randall 1982; Naesje et al. 1986; Shepherd et al. 1986a). Consequently, the current response tests of this study may not be able to readily distinguish between a gradual "drift migration" downstream at night by estuary-bound Nanaimo River chinook fry and a gradual downstream "dispersal" following emergence by stream-type chinooks. In sum, local population factors such as migration distance to nursery areas or estuary size may obscure differences in rheotactic behavior between stream- and ocean-type chinook when testing newly emerged fry. Considerable interpopulation genetic variation in rheotactic behavior exists in newly emerged rainbow trout (*Salmo gairdneri*) and sockeye salmon (*O. nerka*) which maintain residence in freshwater for at least 1 yr (Brannon 1972; Kelso et al. 1981). Alternatively, comparisons of rheotactic behavior over a longer time (e.g. up to 1 yr) may reveal more differences between stream- and ocean-type chinook coinciding with their different ages at seaward migration.

With the exception of major parr mark number, morphological differences between wild chinook fry from the Harrison River and Slim Creek were maintained in the laboratory-reared fry; Slim Creek fry had larger body parts and median fins and were more brightly colored (Table 5). Laboratory-reared chinook fry from Slim Creek had fewer major and total parr marks than did their wild-reared counterparts (1.5 and 7.1 fewer, respectively). Parr mark counts were, however, very similar between laboratory- and wild-reared chinook fry from the Harrison River (0.5 more major and 0.1 fewer total parr marks in the laboratory fry). Consequently, Slim Creek, stream-type chinook fry had only 1.6 more total parr marks than Harrison fry when both were reared in the laboratory compared with a difference of 8.6 for wild fry (Taylor and Larkin 1986). Clearly, a significant environmental component to parr mark number is suggested, since differences between Slim and Harrison fry were minimal when both were incubated and reared in the same laboratory environment. Incubation water temperature regime can alter parr mark counts in chinook fry; lower water temperatures produce higher counts (C. C. Lindsey and E. B. Taylor, unpubl. data). Therefore, water temperature differences in the incubation environments of the Harrison River and Slim Creek (colder) probably account, in large part, for the greater differences in parr mark number in the wild fry.

Despite the clear, inherited differences in morphology between chinook fry from Slim Creek and the Harrison River, these differences could not fully be attributed to a distinction between chinook life history types. While Slim Creek fry had the largest body parts and median fins and were the most colorful fry, fry from both ocean-type populations tended to have larger fins that Eagle River chinook fry (Table 5). Furthermore, Nanaimo River fry were as brightly colored as were fry from the Eagle River. Again, local population factors unrelated to juvenile migration patterns may account for the morphological differences among the four populations. Selection associated with migration distance, water flow levels, and predation have been associated with interpopulation differences in morphology and coloration in stream fishes (Endler 1980; Riddell and Leggett 1981; Taylor and McPhail 1985).

In summary, inherited behavioral and morphological differentiation among populations of juvenile chinook salmon has been demonstrated in this study which supports earlier evidence of genetic divergence among chinook salmon populations (e.g. Ricker 1972; Carl and Healey 1984). At least a portion of this inherited variation may result from a behavioral divergence between stream- and ocean-type life history types. At present it would, however, be premature to describe whole suites of inherited characteristics as either "stream-" or "ocean-type" and to assign them to chinook populations broadly designated as one life history type or the other, particularily if this designation is inferred only from analysis of scales.

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Effects of Cadmium, Copper, and Low pH on Ion Fluxes in the Rainbow Trout, *Salmo gairdneri*

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Reid, S. D., and D. G. McDonald. 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45: 244–253.

Adult and juvenile rainbow trout, *Salmo gairdneri*, acclimated to high and low water Ca levels were statically exposed to equimolor concentrations (~6.5 μmol·L^{-1}) of Cd or Cu at circumneutral pH or pH 4.8 either alone or in metal/H^+ combinations for 24 h. Unidirectional fluxes of Ca^{2+} and Na^+ were measured by means of radiotracers and terminal blood samples were drawn for analysis of Ca^{2+} and Na^+. Plasma Ca^{2+} was found not to be a reliable indicator of disturbances in branchial Ca^{2+} fluxes. Cadmium specifically inhibited Ca^{2+} influx, while having no effect on Ca^{2+} efflux and only minor effects on Na^+ fluxes. The inhibition of Ca^{2+} influx by Cd was modulated by water Ca^{2+} content. Copper altered net Na^+ flux and plasma Na^+ in trout, while having only a transient effect on Ca^{2+} homeostasis. The effects of Cu on the net Na^+ fluxes of trout were not modulated by either water Ca^{2+} or pH. Hydrogen ion perturbed Ca^{2+} dynamics predominantly by stimulating Ca^{2+} efflux, while reducing influx only slightly. Exposure to elevated H^+ (pH 4.8) had no significant effect on net Na^+ balance over the 24-h period. These data indicate significant differences in the toxic mechanisms of three metals with similar ligand binding chemistry.

Des truites arc-en-ciel (*Salmo gairdneri*) adultes et juvéniles acclimatées à des teneurs faibles et élevées en Ca de l'eau ont été exposées de façon statique à des concentrations équimolaires (~6,5 μmol·L^{-1}) de Cd ou de Cu, soit en exclusivité ou en combinaison avec H^+ (pH presque neutre ou pH 4,8). Les flux unidirectionnels de Ca^{+2} et Na^+ ont été quantifiés au moyen d'indicateurs radioactifs; des échantillons de sang ont été prélevés à la fin de l'expérience pour en analyser la teneur en Ca^{+2} et Na^+. La teneur plasmatique en Ca^{+2} n'était pas un indicateur fiable de perturbations des flux de Ca^{+2} dans les branchies. Le Cd a caractéristiquement paralysé l'afflux de Ca^{+2} tout en n'ayant aucune incidence sur la sortie de Ca^{+2} et en ayant une incidence mineure sur les flux de Na^+. L'inhibition de l'afflux de Ca^{+2} par le Cd a été modulée par la teneur en Ca^{+2} dans l'eau. Le Cu a modifié le flux net du Na^+ et la teneur plasmatique en Na^+ chez la truite tout en n'ayant qu'une incidence momentanée sur l'homéostasie du Ca^{+2}. Les incidences du Cu sur les flux nets du Na^+ chez la truite n'ont pas été modulées par la teneur en Ca^{+2} de l'eau ou le pH. La concentration en ions d'hydrogène a bouleversé la dynamique du Ca^{+2} en grande partie par stimulation de la sortie de Ca^{+2} tout en ne réduisant que légèrement l'afflux. L'exposition à une concentration élevée de H^+ (pH 4,8) n'a eu aucune incidence significative sur l'équilibre net du Na^+ pendant la période expérimentale de 24 h. Les présentes données révèlent des différences significatives entre les mécanismes de toxicité de trois métaux possédant des propriétés agglomérantes semblables des ligands.

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Acidification of aquatic environments is often accompanied by elevation of several toxic trace metals. This occurs either because of atmospheric input in the proximity of industrial operations or as a result of dissolution and mobilization from soils and sediments (Beamish and Van Loon 1977). To understand the various interactions of these contaminants, investigators have conducted lethality studies employing mixtures of metals at reduced pH (Brown 1968; Hutchinson and Sprague 1981; Wong et al. 1981). While this approach can be very productive, particularly in providing regulatory information, it is simply not feasible to test all possible combinations for their interactions and toxicity.

Another approach is to examine the mechanism of action of individual toxicants with the aim of eventually predicting how various components of a toxic mixture might interact. Thus, the main purpose of the present study is to contribute to this knowledge by examining how Cu and Cd, arguably the most toxic of the trace metals present in acidified soft waters, affect branchial ion regulation in the rainbow trout, *Salmo gairdneri*. The focus here is on the gills, because they are likely the primary initial target of metal/H^+ toxicity, and on ionoregulation, because this is likely the most sensitive of the physiological functions performed by the gills (cf. McDonald et al. 1986). In the case of the latter, flux rates of Ca^{2+} and Na^+ were studied rather than their respective balances, since disturbances to flux rates are easier to detect than disturbances to body levels, particularly in the case of Ca where the skeleton represents a considerable Ca^{2+} reserve (Dacke 1979; Glowacki et al. 1986; Hobe et al. 1984; Simmons 1971).

Metal exposures were conducted for 24 h only. This avoided possible complications arising from more prolonged exposures such as metal accumulation in various internal organs or induc-

tion of metal-binding proteins. Also, to increase the likelihood of obtaining measurable disturbances, relatively high concentrations of Cu and Cd were employed: 6.5 $\mu mol \cdot L^{-1}$. This concentration is about 20-fold higher than the 96-h LC50 to rainbow trout in soft water for each (Cu, Howarth and Sprague 1978; Cd, Cusimano et al. 1986).

Cadmium and Cu were examined in relation to three variables: water pH, age of the test animals, and water [Ca^{2+}]. The low pH exposure was pH 4.8 or 15.8 $\mu mol\ H^+ \cdot L^{-1}$. This is substantially below the 96-h LC50 for rainbow trout in soft water, pH 4.0 (Graham and Wood 1981), but is a realistic level for natural waters undergoing acidification. Both juvenile and adult rainbow trout were employed, as a recent study (Lauren and McDonald 1986) has shown that electrolyte homeostasis of juveniles is more sensitive to disturbance by metal and acid exposure than that of adults. Furthermore, many of the physiological studies of toxicants used adults whereas lethality studies have been, for the most part, conducted on juveniles. The water Ca variable (i.e. water hardness) was examined for three reasons: (1) acidification is a problem mainly in softwater environments where variations in water Ca can be as important in determining fisheries status as variations in pH (Wright and Snevik 1978); (2) Ca has a well-established protective effect against metal and H^+ toxicity (Alabaster and Lloyd 1980; Brown 1982); and (3) Ca is not only one of the major electrolytes transported by the gills (Perry and Wood 1985), but is also important in the maintenance of the integrity and stability of the branchial epithelium (Chase 1984; Cuthbert and Maetz 1972; McDonald and Rogano 1986; Potts and Fleming 1971). The Ca levels employed were 1.00 and 0.04 $mol \cdot L^{-1}$. These are representative of moderately hard waters, such as the Great Lakes, and extremely soft waters, respectively (NRCC 1981).

Methods and Materials

Experimental Animals

Adult (170–300 g) and juvenile (1.5–2.5 g) rainbow trout were obtained from Spring Valley Trout Farm, Petersburg, Ontario, and Aquafarms of Feversham, Ontario, respectively. The trout were held in large polyethylene tanks supplied with aerated, dechlorinated Hamilton tap water at 11–15°C (Ca^{2+} ~1 $mmol \cdot L^{-1}$, NaCl ~0.6 $mmol \cdot L^{-1}$). Trout were fed regularly with commercial trout pellets. For trout studied in hard water, acclimation commenced 9–12 d prior to experimentation to water of temperature (14 ± 1°C) and composition of that employed in the experiment. Softwater acclimation was accomplished by holding trout in a 400-L temperature-controlled tank, while every other day, the ionic composition was reduced approximately 50% by dilution with deionized tap water. Acclimation of trout to soft water was accomplished over a 30-d period to reduce the stress associated with direct transfer from hard to soft water (McDonald and Rogano 1986). Acclimation water was changed periodically to maintain the ionic composition of the water and reduce the build-up of waste products. Feeding was stopped 4–5 d prior to experimentation.

Test Conditions

Water for acclimation and experimentation was prepared from distilled tap water with appropriate additions of NaCl and $Ca(NO_3)_2$ to reach the desired ionic composition. Water pH was reduced by titration with H_2SO_4. Water chemistry was monitored throughout the exposure periods (Table 1).

TABLE 1. Composition (means, SEM in parentheses) and pH (means, range in parentheses) of the test water. Water samples were taken throughout the exposure period for determination of water chemistry. N = 6; temperature = 13 ± 1°C; all ions in $\mu mol \cdot L^{-1}$.

	Soft water, neutral pH	Hard water, neutral pH	Soft water, pH 4.8	Hard water, pH 4.8
Calcium	30.7	915.0	46.7	1075.0
	(2.4)	(16.5)	(4.4)	(14.5)
Sodium	245.0	255.0	276.0	270.0
	(8.0)	(7.0)	(12.0)	(6.0)
Chloride	274.0	268.0	242.0	268.0
	(20.0)	(12.0)	(5.0)	(12.0)
Potassium	5.1	8.3	6.9	12.1
	(1.5)	(1.0)	(2.8)	(0.4)
Cadmium	6.8	6.5	6.7	6.5
	(0.6)	(0.6)	(0.7)	(0.6)
Copper	6.7	6.2	6.9	5.9
	(0.7)	(0.6)	(0.7)	(0.6)
pH	7.82	7.78	4.77	4.84
	(7.5–8.0)	(7.4–7.9)	(4.6–4.9)	(4.7–5.0)

Test Chambers

Individual adult rainbow trout were contained within acrylic flux chambers, as described by McDonald and Rogano (1986). These rectangular flux chambers (total volume = 5.6 L, flux volume = 2.8 L, internal dimensions (ID) = 17 cm high × 9 cm wide × 37 cm long) were constructed of black acrylic plastic. An inner acrylic chamber (ID = 7 cm high × 5.5 cm wide × 34 cm long), mounted 1 cm off the bottom of the chamber and fitted with a removable lid, served to prevent the fish from turning or rotating in the flux chamber but did not physically restrain it. A multiperforated air line was mounted on the outside of the inner chamber to aerate and mix chamber contents. Juvenile trout were contained in polyethylene containers (total volume = 3.8 L, dimensions = 15.5 cm high × 10.5 cm wide × 10.5 cm long). The containers were cut from sheets of black polyethylene and heat-sealed to provide the final shape. Flux chamber temperature was regulated at 14 ± 1°C. Each container held five fish in 2 L of water and was fitted with an air line for aeration and mixing.

Experimental Protocol

The first series of experiments examined the effects of metals and H^+ on Ca influx and net Na flux in both juveniles and adults. Trout (adults, four fish per treatment; juveniles, three groups of five fish) were allowed to acclimate to the test chambers for 24 h prior to the beginning of an experiment. Experiments were initiated with the addition of ^{45}Ca (6.9–20.6 $kBq \cdot L^{-1}$) to all chambers, while Cd (as 8.9 mM cadmium acetate) or Cu (as 15.7 mM copper sulfate) was added as appropriate. Actual mean exposure concentrations are listed in Table 1. For low pH exposures, acid (as 0.1 N H_2SO_4) was added to reduce the test chamber water to pH 4.8 ($[H^+]$ = 16 $\mu mol \cdot L^{-1}$). After the initial acidification of the water, pH was monitored and readjusted every 15 min for the first 4 h and every 30 min for the remainder of the experiments. Water samples were taken at 0, 2, 4, 8, 12, and 24 h for the determination of net and unidirectional ion fluxes and metal concentrations. Condition of animals was also noted at these times, with any dead trout being removed from their flux chambers to avoid postmortem alteration of water chemistry. All experiments were repeated at least once.

Upon completion of exposure, all fish were killed and weighed. Blood samples were obtained from about one half of the fish. Blood of adult trout was sampled by caudal puncture using a 1-mL syringe and a 22-gauge needle. Juvenile trout were sampled from the caudal peduncle using a 10-μL micropipets. The remainder of the fish were rinsed, frozen, and stored for later analysis of Ca^{2+} uptake (see below). Although all exposures were 24 h in duration, the adult trout were exposed for two periods of 12 h. At the end of the initial 12-h period, the chambers were flushed with fresh water and the second exposure started. However, adult trout exposed to Cd during the initial 12-h flux period were not reexposed during the subsequent flux period.

A second series of experiments were designed specifically to examine the effect of Cd on Na influx in juvenile trout in relation to water pH and Ca^{2+}. In these experiments, groups of 10–15 fish were exposed to a matrix of [Cd] (0, 0.4, 0.8, or 1.6 $\mu mol \cdot L^{-1}$) by pH (7.6 or 5.0) by [Ca^{2+}] (1.0, 0.1, or 0.001 $mmol \cdot L^{-1}$) for 24 h. The experiment was started by the addition of Cd (as $CdCl_2$), 0.1 *N* H_2SO_4 (where appropriate), and 3.7 $kBq \cdot L^{-1}$ of $^{22}Na^+$. At the end of 24 h the animals were collected, killed, weighed, and placed into a sealed 15-mL gamma tube for whole-body counting.

Analytical Techniques

Ion and radiotracer analysis

Concentrations of Na^+, K^+, Ca^{2+}, Cd^{2+}, and Cu^{2+} in plasma and water were measured, after appropriate dilution, by flame atomic absorption spectrophotometry (Varian AA-1275). Cl^- levels were determined by titration with a Buchler–Cotlove chloridometer. Ammonia was measured by a modification of the colorometric method of Verdouw et al. (1978). Radiocalcium activity was detected using liquid scintillation techniques (Beckman LS 230 and a LKB Rackbeta). All activity was corrected for quench, based on quench correction factors generated automatically on the LKB Rackbeta. Radiosodium in tissues and water was measured with a Nuclear Chicago (model 1085) well-type gamma counter.

Flux measurements

Calcium and Na influx rates ($J_{in}^{Ca^{2+}}$ and $J_{in}^{Na^+}$) were calculated based on the accumulation of radioactivity in the body of the fish using the equation

$$J_{in} = C_{wb} \cdot (SA_{wa} \cdot W \cdot t)^{-1}$$

where C_{wb} represents the whole-body radioactivity (disintegrations per minute), SA_{wa} was the radiospecific activity of the water (disintegrations per minute per micromole), W was the weight of the fish (kilograms or grams), and t was the total time (hours) of exposure to the radiotracer. Backflux correction was determined to be unnecessary based on the body and water specific activity. Water specific activity was virtually constant throughout the exposures.

Whole body $^{45}Ca^{2+}$ activity of the adult trout was determined using the in vivo Ca^{2+} uptake method described by Perry and Wood (1985). Briefly, the method entailed rinsing trout carcasses for 2 min in flowing well water (10 $mmol \cdot L^{-1}$) to displace adsorbed $^{45}Ca^{2+}$ from the body surface. The carcasses were weighed and homogenized with an additional 100 mL of distilled water in a Waring blender. Quadruplicate aliquots (0.4–0.8 g) of the homogenate from each fish were digested overnight at 50°C in tissue solubilizer (NCS, Amersham). The digests were then neutralized with acetic acid and diluted with 10 mL of scintillation fluid (OCS, Amersham) prior to counting. A similar approach was used to determine the juvenile trout $^{45}Ca^{2+}$ C_{wb}. Juvenile trout were digested for 2–3 d in 5 mL of 10 *N* KOH. Five microlitres of digest was diluted to 5 mL with deionized water followed by the addition of 300 μL of glacial acetic acid, 1 mL of triton *X*-114 (BDH Chemical), and 10 mL of ACS (Amersham Corporation). Quadruplicate samples were prepared for each fish. Whole-body $^{22}Na^+$ activity of juvenile trout was determined by counting the whole fish in the gamma counter.

Net fluxes (J_{net}) of Na^+ and Ca^{2+} (micromoles per kilogram per litre) were calculated from changes in their water concentrations. Calcium efflux (J_{out}) was calculated from the difference between J_{net} and J_{in}.

TABLE 2. Percent mortality in juvenile rainbow trout (1.5–2.5 g) exposed to Cd, Cu, or H^+ alone or in metal/H^+ combinations for 24 h. Concentrations of metals were 6 $\mu mol \cdot L^{-1}$; water pH in H^+ was 4.8 (control pH = 7.8); sample size for each treatment at start of exposure was 20–21 trout.

	Control	H^+	Cd	Cd/H^+	Cu	Cu/H^+
Hard water (Ca^{2+} = 1 $mmol \cdot L^{-1}$)	0.0	0.0	28.6	0.0	71.4	40.9
Soft water (Ca^{2+} = 0.04 $mmol \cdot L^{-1}$)	0.0	0.0	34.8	19.0	82.6	52.4

Statistics

All data presented are means ± 1 SEM. Statistical differences between treatments were analyzed by ANOVA and a Student–Newman–Keuls ranking test for means of equal or unequal sample size (Rosner 1982). Significance was set at a 95% level of confidence.

Results

Exposures

Despite the relatively high loading densities in these experiments (adults = 80 $g \cdot L^{-1}$, juveniles = 5 $g \cdot L^{-1}$) compared with those recommended for static toxicity bioassays (1 $g \cdot L^{-1}$, Sprague 1969), metal concentrations remained stable throughout the exposures (means in Table 1) and ammonia levels rose to no more than 390 $\mu mol \cdot L^{-1}$. The latter were unlikely to have had toxic effects, as the final values represented less than 20% of the 96-h LC50 of ammonia for rainbow trout (0.53 $mg \cdot L^{-1}$, Arthur et al. 1987) at the water pH's and temperature employed (Emerson et al. 1975). Equally important was the lack of any significant change in Na^+ influx or efflux in control animals, over the course of the exposures, which suggests that ammonia accumulation was below the threshold to cause ionoregulatory disturbances.

Mortality

In terms of mortality, adult trout were more resistant that juveniles. No adults died during either holding, softwater acclimation, or metal/H^+ exposure. In juveniles, there was less than 4% mortality during holding and acclimation, but significant mortality occurred with both Cd and Cu exposure, with the latter being more toxic (Table 2). Reduction of Ca^{2+} in the water tended to increase metal toxicity. However, in both hard and soft water, the addition of H^+ appeared to reduce metal toxicity.

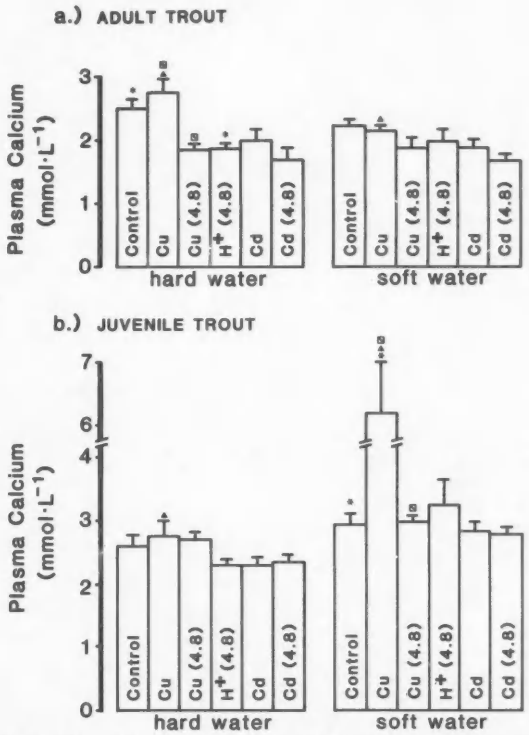

FIG. 1. Plasma Ca^{2+} concentrations of rainbow trout following 24 h of exposure to 6.5 μmol Cd or Cu·L⁻¹ at circumneutral pH (7.6) or pH 4.8. Animals were acclimated and exposed in either hard water ($[Ca^{2+}]$ = 1 mmol·L⁻¹) or soft water ($[Ca^{2+}]$ = 0.03 mmol·L⁻¹). Data are presented as means ± 1 SEM. Statistical comparisons, using ANOVA and a Student–Newman–Keuls ranking test at a 95% level of confidence, have been depicted to illustrate significant differences due to (1) metal treatment, (2) water Ca^{2+} content, or (3) water pH. Significantly different data are indicated with matched pairs of symbols. (a) Plasma levels of adult trout (150–300 g); N = 8 for each treatment. (b) Plasma concentrations of juveniles (1.5–2.5 g); N = 5 for each treatment.

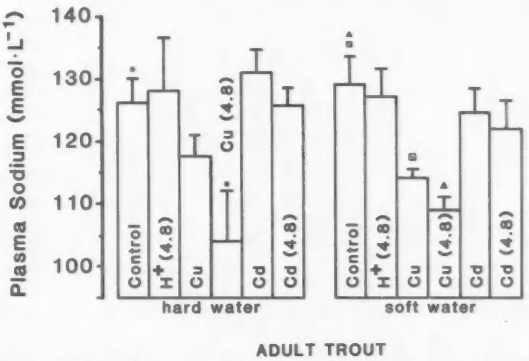

FIG. 2. Plasma Na^{+} concentrations of adult rainbow trout following 24 h of exposure to 6.5 μmol Cd or Cu·L⁻¹ at circumneutral pH (7.6) or pH 4.8. Animals were acclimated and exposed in either hard water ($[Ca^{2+}]$ = 1 mmol·L⁻¹) or soft water ($[Ca^{2+}]$ = 0.03 mmol·L⁻¹). Data are presented as means ± 1 SEM; N = 6 for each treatment. Statistical comparisons, using ANOVA and a Student–Newman–Keuls ranking test at a 95% level of confidence, have been depicted to illustrate significant differences due to (1) metal treatment, (2) water Ca^{2+} content, or (3) water pH. Significantly different data are indicated with matched pairs of symbols.

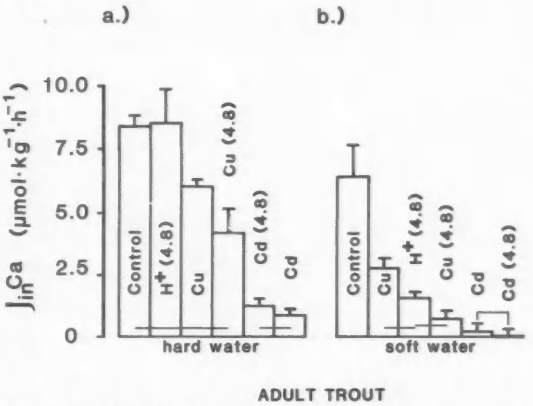

FIG. 3. Mean Ca^{2+} influxes ($J_{in}^{Ca^{2+}}$) of adult rainbow trout over 12 h of exposure to 6 μmol Cd or Cu·L⁻¹ at circumneutral pH (7.6) or pH 4.8 (4.8). Data are means ± 1 SEM; N = 8 for each treatment. The horizontal lines connect bars which are not significantly different from one another at a confidence limit of 95% (ANOVA). (a) Exposures of hardwater-acclimated animals in hard water ($[Ca^{2+}]$ = 1 mmol·L⁻¹); (b) softwater-acclimated animals exposed in soft water ($[Ca^{2+}]$ = 0.03 mmol·L⁻¹).

Changes in Plasma Electrolytes

Measurements of both $[Ca^{2+}]$ and $[Na^{+}]$ were made on plasma of adult trout (Fig. 1 and 2), but on juveniles, only the former were made because of the small sample volumes (2–5 μL) obtained. Furthermore, the small sample volume probably contributed to the variability of those data (Fig. 1). For example, one softwater-acclimated juvenile exposed to Cu (Fig. 1b) had a plasma Ca level of 12.6 mmol·L⁻¹. This could be real and due, for example, to hemoconcentration secondary to NaCl loss (McDonald 1983a), but a value this high is more likely a sampling artifact.

Despite these variations in the data, it is apparent that 24-h metal/H^{+} exposures had relatively little effect on plasma electrolytes. The only major disturbance was a reduction in plasma $[Na^{+}]$ in adults exposed to Cu (Fig. 2). Once these data were excerpted, no significant effect remained of water Ca^{2+} level, water pH, or Cd on plasma $[Na^{+}]$ in adults or plasma $[Ca^{2+}]$ in both adults and juveniles. Furthermore, plasma $[Ca^{2+}]$ was virtually identical in adults and juveniles. Plasma $[Na^{+}]$ averaged 126.7 ± 0.37 mmol·L⁻¹ (N = 48, hardwater and softwater adults combined) and plasma $[Ca^{2+}]$ averaged 2.48 ± 0.066 mmol·L⁻¹ (N = 156, hardwater and softwater adults and juveniles combined.

Calcium Influx

In control animals (neutral pH, no metals) the rate of Ca uptake ($J_{in}^{Ca^{2+}}$) was relatively unaffected by either age or ambient $[Ca^{2+}]$. In low Ca^{2+} ($[Ca^{2+}]$ = 37 μmol·L⁻¹ for 30 d), $J_{in}^{Ca^{2+}}$ was 6.4 ± 0.56 nmol·g⁻¹·h⁻¹ in juveniles and 6.4 ± 1.24 μmol·kg⁻¹·h⁻¹ in adults. In high Ca^{2+} (1000 μmol·L⁻¹ for >30 d), $J_{in}^{Ca^{2+}}$ was 7.1 ± 0.69 nmol·g⁻¹·h⁻¹ in juveniles and 8.4 ± 0.44 μmol·kg⁻¹·h⁻¹ in adults.

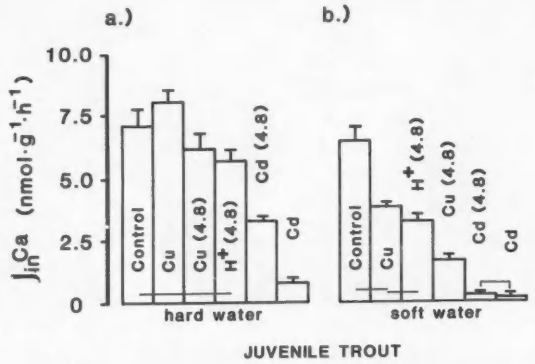

FIG. 4. Mean Ca^{2+} influxes ($J_{in}^{Ca^{2+}}$) of juvenile rainbow trout over 24 h of exposure to 6.5 μmol Cd or Cu·L⁻¹ at circumneutral pH (7.6) or pH 4.8. Data are means ± 1 SEM; $N = 15$ for each treatment. The horizontal lines connect bars which are not significantly different from one another at a confidence limit of 95% (ANOVA). (a) Hardwater-acclimated animals exposed in hard water ($[Ca^{2+}] = 1$ mmol·L⁻¹); (b) softwater-acclimated animals exposed in soft water ($[Ca^{2+}] = 0.03$ mmol·L⁻¹).

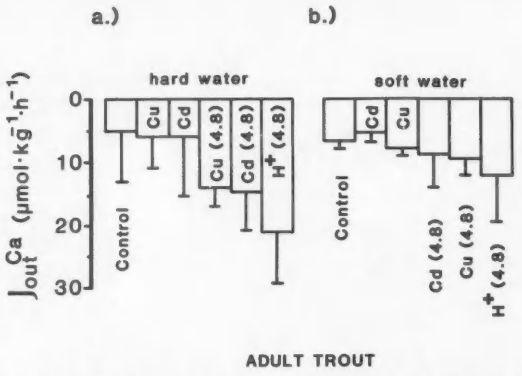

FIG. 5. Mean Ca^{2+} effluxes ($J_{out}^{Ca^{2+}}$) of adult rainbow trout over 24 h of exposure to 6.5 μmol Cd or Cu·L⁻¹ at circumneutral pH (7.6) or pH 4.8. Data are means ± 1 SEM; $N = 6$ for each treatment. (a) Hardwater-acclimated animals exposed in hard water ($[Ca^{2+}] = 1$ mmol·L⁻¹); (b) softwater-acclimated animals exposed in soft water ($[Ca^{2+}] = 0.03$ mmol·L⁻¹).

With a few exceptions noted below, all metal and H^+ exposures caused a significant inhibition of $J_{in}^{Ca^{2+}}$ in adults and juveniles relative to controls. In Fig. 3 and 4, the average influx values over the 24-h exposure period are shown ranked according to the degree of inhibition. Regardless of age of animal or Ca^{2+} acclimation, Cd was more inhibitory than either Cu or low pH. Indeed, Cu exposure had no effect on $J_{in}^{Ca^{2+}}$ in hardwater-acclimated juveniles (Fig. 4a).

In both adults and juveniles, water Ca^{2+} provided significant protection against the effects of Cd, Cu, and H^+. The degree of inhibition of $J_{in}^{Ca^{2+}}$ relative to controls was much greater in softwater-acclimated animals than in hardwater animals (Fig. 3a vs. 3b; Fig. 4a vs. 4b).

The effects of low pH exposure were somewhat more complicated than those of either Cu or Cd. By itself, low pH exposure had a relatively small effect, particularly in hard water (Fig. 3a, 4a). Where inhibition by H^+ did occur (Fig. 3b, 4b), it was similar in magnitude than that of Cu alone. Consequently, when the two were combined, the effect was additive although the degree of inhibition was less than the sum of the two individual effects. In contrast, low pH had very little further effect when combined with Cd and in one case (hardwater-acclimated juveniles; Fig. 4a) appeared to be protective.

Calcium Efflux

The maintenance of electrolyte balance depends not only on the rate of influx but also on the rate of loss, or efflux. Figure 5 shows efflux data calculated from net flux and influx values for adult trout. These values were quite variable, particularly in hard water where small changes in Ca concentration were being measured against a large background. As a result, there were no statistically significant differences amongst the various treatments. Nonetheless, the data suggest that of the various exposures, only the low pH treatment stimulated Ca^{2+} efflux. When low pH was combined with metals, the stimulation of Ca^{2+} efflux by H^+ was slightly reduced. Alone, neither metal exposure nor changes in water Ca^{2+} appeared to alter $J_{out}^{Ca^{2+}}$ which overall averaged 5.9 ± 0.9 μmol·kg⁻¹·h⁻¹.

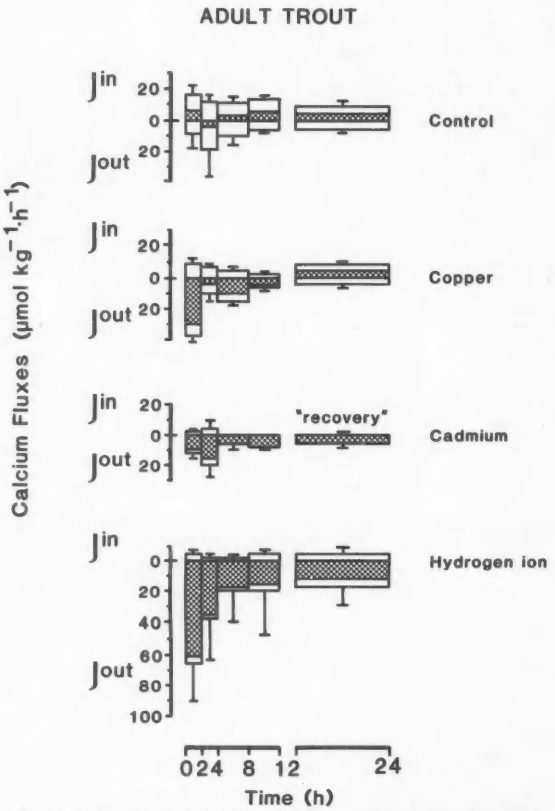

FIG. 6. Unidirectional Ca^{2+} fluxes (J_{in} and J_{out}, J_{net} indicated by stipled areas) of softwater-acclimated adult trout over the two 12-h exposure periods (controls, pH 7.8; Cu, 6 μmol·L⁻¹; Cd, 6.5 μmol·L⁻¹; H^+, pH 4.8). Data are means ± 1 SEM; $N = 6$ for all treatments. "Recovery" indicates that during this period, Cd^{2+} was not reintroduced into the flux chambers, i.e. fish were allowed to recover from the initial 12 h of Cd exposure.

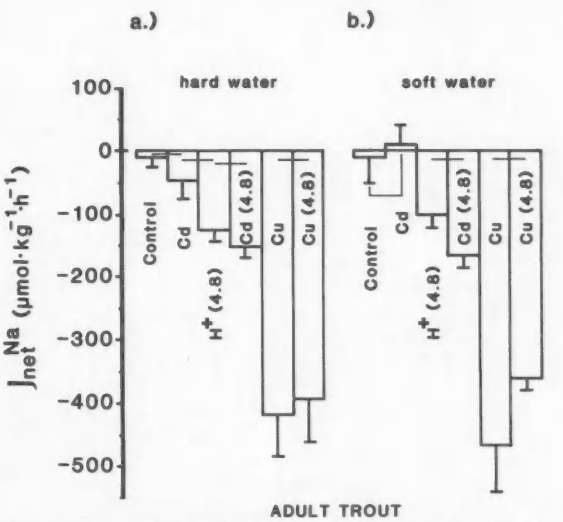

FIG. 7. Mean net Na^+ fluxes ($J_{net}^{Na^+}$) of adult rainbow trout over 24 h of exposure to 6.5 μmol Cd or Cu·L^{-1} at circumneutral pH (7.6) or pH 4.8. Data are means ± 1 SEM; N = 8 for each treatment. The horizontal lines connect bars which are not significantly different from one another at a confidence limit of 95% (ANOVA). (a) Hardwater-acclimated animals exposed in hard water ([Ca^{2+}] = 1 mmol·L^{-1}); (b) softwater-acclimated animals exposed in soft water ([Ca^{2+}] = 0.03 mmol·L^{-1}).

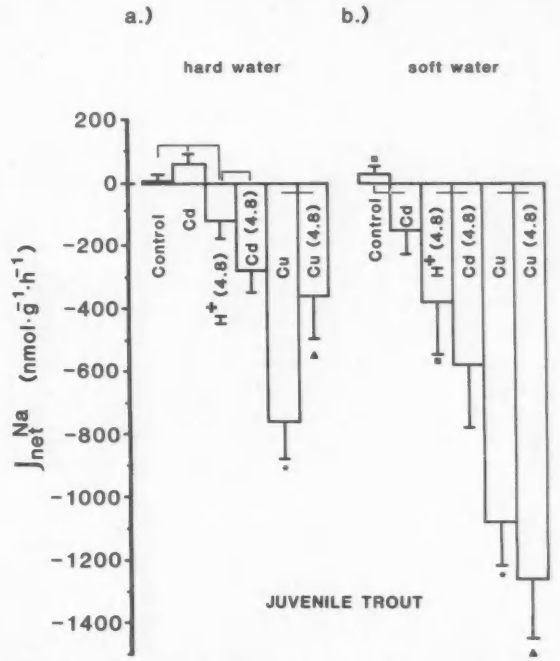

FIG. 8. Mean net Na^+ fluxes ($J_{net}^{Na^+}$) of juvenile rainbow trout over 24 h of exposure to 6.5 μmol Cd or Cu·L^{-1} at circumneutral pH (7.6) or pH 4.8. Data are means ± 1 SEM; N = 10 for each treatment. Statistical comparisons, using ANOVA and a Student–Newman–Keuls ranking test at a 95% level of confidence, have been depicted to illustrate significant differences due to (1) metal treatment, (2) water Ca^{2+} content, or (3) water pH. Significantly different data are indicated with matched pairs of symbols. The horizontal lines connect bars which are not significantly different from one another at a confidence limit of 95% (ANOVA). (a) Hardwater-acclimated animals exposed in hard water ([Ca^{2+}] = 1 mmol·L^{-1}); (b) softwater-acclimated animals exposed in soft water ([Ca^{2+}] = 0.03 mmol·L^{-1}).

Persistence of the Disturbance

The 24-h averages for $J_{in}^{Ca^{2+}}$ and $J_{out}^{Ca^{2+}}$ (Fig. 3–5) reveal marked differences amongst the various treatments. Even further differences are revealed when the development of the metal/H^+ effects over time is examined. These data are shown in Fig. 6 for adult trout acclimated to soft water. Similar data were obtained for hardwater animals, but the effects were generally less (see Fig. 3) and somewhat more variable, as discussed above. As Fig. 6 illustrates, most of the variation in time with unidirectional Ca^{2+} flux rates can be attributed to the metal/H^+ exposures, since J_{in} and J_{out} in controls changed little during the 24-h exposure period.

When fish were Cu-exposed, $J_{in}^{Ca^{2+}}$ gradually declined over the initial 12 h of the experiment. $J_{out}^{Ca^{2+}}$ was initially stimulated but this response diminished over time and the average $J_{out}^{Ca^{2+}}$ was not significantly different from controls. Upon reexposure, the trout were able to reestablish a positive $J_{net}^{Ca^{2+}}$ identical to that of the controls even though the metal was still present.

When fish were exposed to low pH, $J_{in}^{Ca^{2+}}$ was immediately inhibited and $J_{out}^{Ca^{2+}}$ stimulated, leading to net Ca^{2+} losses. Initially, $J_{out}^{Ca^{2+}}$ increased 13-fold but gradually decreased during the first 12 h until stabilizing (at +4 h) at a level about twice the control rate. During the final 12 h of exposure, the H^+-exposed fish continued to lose Ca due to a slight reduction in influx and a stimulation of efflux.

Cadmium did not initially disrupt $J_{in}^{Ca^{2+}}$; rather, it took approximately 4 h before significant inhibition was evident. Furthermore, Cd had no significant effect on $J_{out}^{Ca^{2+}}$. During the final 12-h flux period, Cd was not reintroduced into the flux chambers. Although the Cd concentration was reduced to 91% of the initial exposure concentration (0.54 ± 0.066 μmol·L^{-1}) by flushing with Cd-free water, $J_{in}^{Ca^{2+}}$ remained significantly inhibited.

Sodium Fluxes

The effects of Cd, Cu, and low pH on $J_{net}^{Na^+}$ over 24 h are shown in Fig. 7 and 8. Control fish from both hard and soft water were found to be in Na^+ balance, with net fluxes not significantly different from zero. Exposure to Cd alone had little effect on Na^+ balance in either adults (Fig. 7) or juveniles (Fig. 8). In both hard and soft water, H^+ exposure led to net losses of Na^+. These losses, although significantly different from zero, were not significantly different from controls. In contrast, Cu caused significant losses of Na^+ which were similar in hard and soft water. In adults these losses were 420 ± 65 and 465 ± 75 μmol·kg^{-1}·h^{-1}, respectively, and in juveniles were found to be approximately twice as great at 760 ± 120 and 1080 ± 140 μmol·kg^{-1}·h^{-1}. Combining the metals with low pH did not significantly increase net Na^+ losses beyond those caused by exposures to the metals alone.

The effects of Cd and low pH on Na^+ influx ($J_{in}^{Na^+}$) in juveniles are shown in Fig. 9. In this experiment a range of Cd concentrations up to 1.6 μM was used, the maximum being about five times the 96-h LC50. Despite this, there were no

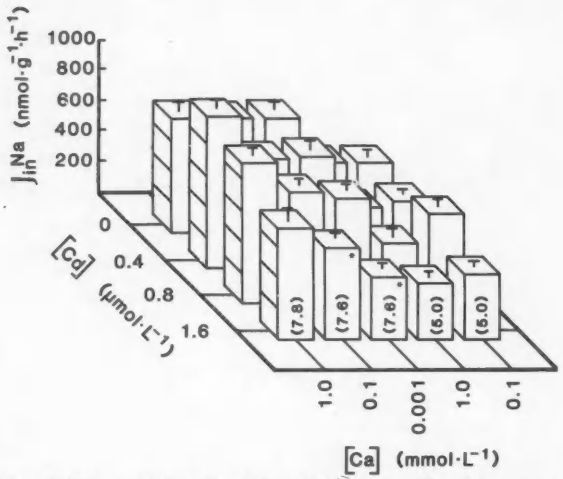

FIG. 9. Effect of Cd on Na^+ influx ($J_{in}^{Na^+}$) in juvenile rainbow trout at different pH and water $[Ca^{2+}]$. Data are presented as means ± 1 SEM; $N = 10–14$. Asterisks indicate means significantly different from those animals at the same pH and $[Ca^{2+}]$ which were not exposed to Cd.

TABLE 3. Comparison of ion flux and turnover rates for rainbow trout. Data are from the present study except where footnoted.

	Juveniles	Adults
Weight (g)	1.5–2.5	170–300
$J_{in}^{Ca^{2+}}$ (nmol·g⁻¹·h⁻¹)	6.8	7.8
Whole-body Ca^{2+} (μmol·g⁻¹)	86[a]	125[a]
Daily turnover (%)	0.19	0.15
$J_{in}^{Na^+}$ (nmol·g⁻¹·h⁻¹)	712	320[b]
Whole-body Na^+ (μmol·g⁻¹)	67[a]	56[a]
Daily turnover (%)	25.8	13.7
Gill surface area (cm²·g⁻¹)	2.73[c]	1.99[c]

[a]Shearer 1984.
[b]Lauren and McDonald 1985.
[c]Hughes and Morgan 1973.

mortalities over the 24-h exposure. At circumneutral pH, $J_{in}^{Na^+}$ averaged 712 ± 26 nmol·g⁻¹·h⁻¹ and was not influenced by external Ca^{2+}. At pH 5.0, $J_{in}^{Na^+}$ was reduced by about 55% and averaged 319 ± 26 nmol·g⁻¹·h⁻¹, and again no influence of external Ca^{2+} was apparent. Cadmium had no significant effect on $J_{in}^{Na^+}$ except at the highest concentration tested (1.6 μmol·L⁻¹) at pH 7.6. Here, water Ca had a protective effect; this is, the inhibition by 1.6 μmol Cd·L⁻¹ was progressively greater with declining Ca concentrations. At pH 5.0 and 1.6 μmol Cd·L⁻¹, no significant inhibition was observed beyond that caused by pH 5.0 alone.

Discussion

Although the characteristics of Na and Ca regulation have been studied in the rainbow trout (e.g. Kerstetter et al. 1970; Wood and Randall 1973; Perry and Wood 1985; McDonald and Rogano 1986), this is the first study to examine Na^+ and Ca^{2+} fluxes in both adults and juveniles. As a result, we are able to offer some new insights into developmental changes in ion regulation. Table 3 lists a comparison of influx rates for both ions together with turnover rates calculated using whole-body ion contents (after Shearer 1984). This analysis reveals two important points. Firstly, Na^+ turnover (as a percent of whole-body Na^+) is about 100-fold higher than Ca^{2+} turnover in both juveniles and adults, thus indicating the much greater exchangeability of the Na^+ pool. Secondly, turnover rates for both ions decrease with age. The drop is greater for Na^+ than Ca^{2+} and is nearly 50% in the former. This may be the result of a decrease in branchial ion permeability or may simply reflect a reduction in the weight-specific surface area of the gills with growth (Morgan 1971). In either case, the phenomenon of decreasing ion turnover may at least partially explain the decreased sensitivity to toxicants with age. In the present study, this was evident both as smaller net ion losses for the same severity of exposure (Fig. 7 vs. Fig. 8) and in a much reduced mortality (Table 2). However, the important point is that the differences were mainly of a quantitative nature, i.e. the same basic physiological responses were seen in juveniles and adults. This means that the toxic mechanisms of metal/H^+ may well be independent of growth at least over the size range 1.5–300 g.

This study has clearly shown that Cd, Cu, and H^+ can each disrupt transepithelial ion exchange in the rainbow trout. However, the more important observation is that the effects of each were different from one another with respect to specific site of action, rapidity of action, and persistence of the disturbance. Furthermore, Cu and H^+ had only minor effects on Ca^{2+} balance and major effects on Na^+ balance whereas the reverse was the case for Cd.

In the ensuing discussion, we have assumed that the primary site of action of the metals is the gills, at least over the short term of exposure employed here. For the disturbances to Na^+ balance, this can be accepted with little question, since it is well established that the gills are the primary route for both uptake and loss of this ion (McDonald 1983a). This is perhaps less certain for Ca^{2+}. Potential extrabranchial sites of Ca exchange include the intestine, the general body surface (scales), and the kidneys. Calcium absorption by the body surface has been shown to exist (Dacke 1979; Simmons 1971; Perry and Wood 1985), but the exact mechanism(s) and relative importance in Ca balance have not been determined. Intestinal uptake of Ca has also been shown to exist (Dacke 1979), but this may be relatively unimportant, since nonfeeding fish can satisfy their Ca requirements by direct branchial uptake (Ichii and Mugiya 1983), and even when feeding, only 35–50% of the total absorbed Ca is of dietary origin (McCay et al. 1936; Berg 1968; Ichii and Mugiya 1983). The precise importance of renal Ca loss in Ca homeostasis has also yet to be determined. Nonetheless, renal losses are likely second only in magnitude to losses across the gills. Under normal circumstances, renal Ca loss can amount to 50% of the total whole-body efflux (Hobe et al. 1984). However, Giles (1984) reported no significant change in renal electrolyte handling during 178 d of exposure to sublethal Cd levels. This tends to suggest that while renal Ca losses may be significant, their contribution to the total loss is unlikely to increase in the face of metal/H^+ exposures.

The gill surfaces of fish, by analogy with other transport epithelia, are likely to contain a variety of complex organic anionic ligands which can probably act as cationic binding sites. Consequently much of the toxic action of metals and H^+ can likely be interpreted in terms of gill surface interactions (Pagenkopf 1983; McDonald et al. 1986). Factors important for such interactions could then be any one or more of the following: the metal's formal charge, ionic radius, donor ligand preference, tendency to form covalent complexes, and binding affin-

ity. If these factors are considered, then it is possible to explain why the branchial effects of Cd, Cu, and H^+ are different from one another.

Action of Cadmium

Cadmium appears to be much more specific in its action than either H^+ or Cu, inhibiting Ca uptake in a persistent fashion (Fig. 6) while having little effect on Ca^{2+} efflux (Fig. 5), net Na^+ flux (Fig. 8), or Na^+ influx (Fig. 9). In a variety of circumstances, Cd has been shown to be a highly specific inhibitor of Ca-binding proteins. It is a blocker of Ca^{2+} channels in neurons (Giles et al. 1983) and has a higher binding affinity than Ca for calmodulin (Lock et al. 1984; Richardt et al. 1986; Suzuki et al. 1985). Calmodulin has been found in the cell fraction of the gills containing the high-affinity Ca^{2+}-ATPase thought to be responsible for Ca influx (Flik et al. 1985). Therefore, if the branchial uptake of Ca is calmodulin dependent, then its blockade could easily lead to a disruption of Ca transport.

The specific biochemical nature of the action of Cd on the gills has not been resolved. Studies by Bansal et al. (1985) showed that Cd inhibited a Ca^{2+}-ATPase isolated from the gills and heart of the freshwater teleost *Saccobrancus fossilis* but only at concentrations greater than 500 μM; at lower concentrations, Cd was stimulatory. This is a somewhat contradictory finding, since we show that Cd at 6 μM inhibits Ca uptake in vivo. However, the explanation may be the particular ATPase examined. As Flik et al. (1985) pointed out, there are both high- and low-affinity ATPase's present in the gills, and only the former is thought to play a role in Ca transport.

Action of Copper

Copper had very little effect on Ca transport relative to Cd. This somewhat surprising, since both are similar in toxicity (96-h LC50 = 0.3 μM, cf. McDonald et al. 1986), were used in equimolar concentrations (6 $\mu mol \cdot L^{-1}$), and have similar chemistries with regard to their tendency to form covalent complexes or ion pairs with ligands (Nieboer and Richardson 1980). Although perhaps oversimplified, their respective ionic radii may be the key to the difference in their effects. Cadmium has a very similar ionic radius to that of Ca (1.14 vs. 1.18 Å (1Å = 0.1 nm)) whereas Cu is much smaller (0.72–0.96 Å). Binding ligands that are highly specific for Ca are therefore much more likely to bind Cd than Cu.

Copper, on the other hand, is highly toxic to Na transport. For example, Cu at 1.6 μM caused 72% inhibition of $J_{in}^{Na^+}$ in juvenile rainbow trout (Lauren and McDonald 1986) whereas Cd at 1.6 $\mu mol \cdot L^{-1}$ (Fig. 9) caused only 20% (at $[Ca^{2+}] = 0.1$ $mmol \cdot L^{-1}$) or only 3% inhibition (at $[Ca^{2+}] = 1.0$ $mmol \cdot L^{-1}$). Indeed, Cu concentrations as low as 0.2 $\mu mol \cdot L^{-1}$ have been shown to inhibit Na influx in rainbow trout (Lauren and McDonald 1985), an effect that has been shown both in vitro (Lorz and McPherson 1976; Riedel and Christensen 1979) and in vivo (Lauren and McDonald 1987b) to be due to the inhibition of branchial Na^+/K^+-ATPase. Based on kinetic measurements, Lauren and McDonald (1987a) argued that the inhibition was of a noncompetitive nature, due, perhaps, to damage of the ATPase (e.g. catalysis of sulphydryl bridge formation) rather than to competitive Cu binding to the Na^+/K^+ binding site.

Copper also has a much greater effect on the ionic permeability of the gills than Cd. This is seen as the greatly increased net Na loss relative to Cd (Fig. 7, 8). This stimulation of efflux is thought to be the result of the weakening of the intercellular tight junctions brought on by the displacement of membrane-bound Ca^{2+} (McWilliams 1983; McDonald 1983b; Lauren and McDonald 1985). Since the intercellular (paracellular) pathway is not thought to be particularly ion specific (Marshall 1985), one might expect that Cu should also increase $J_{out}^{Ca^{2+}}$. The absence of this occurrence (Fig. 5) might be explained if Ca^{2+} and Na^+ diffuse by a different route across the gills, but the simplest explanation may be the marked difference between Ca^{2+} and Na^+ in their respective transbranchial diffusion gradients. The ratio of blood to water concentrations for Na^+ in the present study was greater than 250:1, while for Ca^{2+} it was only about 40:1 in soft water and 1:1 in hard water (calculated assuming that 57% of blood Ca is ionic; Andreasen 1985).

If Cu increases ionic permeability because of the displacement of Ca from surface ligands, then one might expect Cd to have a similar effect. Since it did not, Fig. 5a might be interpreted as meaning that Ca^{2+} plays less of a role in gill membrane stability than has been hypothesized. Alternatively, Cd may act in some respects as a Ca agonist, replacing Ca but having similar stabilizing effects on membranes.

Action of Hydrogen Ions

The present study shows that the action of H^+ is intermediate in many respects to that of Cu and Cd. Hydrogen ions have a somewhat greater effect than Cu on Ca influx and efflux and a smaller effect on Na fluxes. In this regard, one can conclude that H^+ are relatively weaker and less specific in their action upon the gills than either Cu or Cd. In fact, the effects of H^+ on the gills are potentially quite numerous and include nonspecific binding to surface ligands, competitive interactions with Ca^{2+}, Na^+, and metals for surface ligands (Nieboer and Richardson 1980), and alteration of metal speciation (Campbell and Stokes 1985). This competition is evident in the case of Cd where the inhibition of $J_{in}^{Ca^{2+}}$ (Fig. 4a) and $J_{in}^{Na^+}$ (Fig. 9) was substantially less at pH 5.0 than at pH 7.6. No such effect was seen in the case of Cu; instead, H^+ and Cu were seen to be less than fully additive in their effects. The absence of a full additive interaction could be because Cu has a much higher binding affinity than H^+ for various ligands or because of speciation changes in Cu from pH 7.6 to pH 5.0. At pH > 7.0, a significant fraction of the total soluble Cu will be in the form of the first hydrolysis species of Cu ($CuOH^+$), which is presumably less surface active, whereas at pH 5.0 or less, Cu will be entirely in the form of the free ion, Cu^{2+}.

Nonetheless, the competitive interactions of H^+ are important with regard to both Cu and Cd. Lowering pH has been shown to both reduce the toxicity of both Cu and Cd to rainbow trout (Table 2; also Cusimano et al. 1986) and also to reduce the uptake of both metals (McDonald et al. 1986; Lauren and McDonald 1986).

Conclusions

On the basis of this study, we can now draw three conclusions which should be useful in predicting toxic metal interactions in low pH environments.

(1) Overall, Cd appears to have a somewhat smaller impact on branchial ionoregulation than Cu, at least over the 24-h period examined here. Nonetheless, these metals have similar acute toxicities (i.e. 96-h LC50's). This apparent discrepancy may either be attributable to the greater persistence of the action

of Cd relative to Cu, i.e. the branchial effects of Cd become more severe with time, or that Cd exerts a greater proportion of its toxic action internally (e.g. via neural or renal dysfunction).

(2) Water Ca (i.e. water hardness) has a fundamentally different role to play in the toxicity of Cd compared with Cu. Calcium and Cd are clearly antagonistic whereas Ca has relatively little influence on the toxic action of Cu.

(3) Reduction of water pH modifies the toxic effects of Cu and Cd in a largely protective fashion. However, this protection is illusory because it is the acidification process which is primarily responsible for increased metal levels in the first place.

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Effects of Acid pH Shock on Phosphate Concentrations and Microbial Phosphate Uptake in an Acidifying and a Circumneutral Lake[1]

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Nalewajko, C., and M. A. O'Mahony. 1988. Effects of acid pH shock on phosphate concentrations and microbial phosphate uptake in an acidifying and a circumneutral lake. Can. J. Fish. Aquat. Sci. 45: 254–260.

Water samples from Plastic Lake and St. Nora Lake, Dorset, Ontario, were subjected to a rapid decrease in pH (acid pH shock). In both lakes, phosphate concentrations changed following pH shock. Minimal phosphate concentrations were observed in the pH 5.2–5.8 range. Maximal concentrations in both lakes occurred at pH 4.0: in Plastic Lake the mean increase at pH 4 was 3.0 times ambient and in St. Nora Lake was 2.5 times ambient. Particles >0.45 μm were the major source of the phosphate released on acid pH shock. Rate constants of $^{32}PO_4$ uptake by microorganisms decreased in response to acid pH shock, and bacteria were affected more than algae; the decrease in rate constants was more severe in Plastic than in St. Nora Lake but we could not distinguish the relative contributions of H^+ toxicity and isotope dilution (by the extra phosphate released) to the observed decline.

On a soumis des échantillons d'eau recueillis dans les lacs Plastic et St. Nora (Dorset, Ontario) à une chute rapide du pH (choc acide). Dans les deux lacs, les concentrations de phosphate ont varié par suite du choc acide; on a observé des concentrations minimales de phosphate quand le pH était de 5,2 à 5,8. Dans les deux lacs, les concentrations maximales de phosphate ont été observées au pH 4,0 : dans le lac Plastic, l'augmentation moyenne au pH 4 s'élevait à 3,0 fois la concentration ambiante tandis que dans le lac St. Nora, elle se situait à 2,5 fois la concentration ambiante. Les particules de taille supérieure à 0,45 μm étaient la principale source de phosphate relâché au moment du choc acide. Les paramètres du taux de captation du $^{32}P\text{-}PO_4$ par les microorganismes ont accusé une baisse en réaction au choc acide; ainsi les bactéries ont été plus touchées que les algues. La baisse des paramètres de taux était plus marquée dans le lac Plastic que dans le lac St. Nora mais on n'a pu identifier les contributions relatives de la toxicité du H^+ et de la dilution des isotopes (par le lâcher de plus grandes concentrations de phosphate) à la baisse observée.

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In lakes that are undergoing acidification the pH does not decrease at a constant rate; instead, it decreases abruptly in response to acid precipitation events, and at snowmelt, but increases somewhat at other times. The lake biota is thus subjected to a range of scales of pH change. Superimposed on the trend of decreasing mean annual pH over tens of years are short-term pH shocks, on the order of months, days, and hours.

The effect of lake acidification on phosphorus–phytoplankton dynamics is not well known. A decrease in pH may bring H^+ concentrations into the toxic range for some planktonic microorganisms, and may exert an indirect effect as well, mediated by changes in the chemical species and concentrations of important nutrients. Speciation of phosphate may not pose serious problems because the H_2PO_4 form predominates in the pH 2.5–7 range, but binding by aluminum, iron, manganese, and calcium, particularly in the pH 5–6 range, is likely to decrease phosphorus concentrations (Stumm and Morgan 1970; Dickson 1978). Experimental acidification of Lake 223 (Experimental Lakes Area, northwestern Ontario) did not decrease phosphorus concentrations (Schindler 1980), and reports that total phosphorus and soluble reactive phosphorus concentrations are lower in acidifying compared with circumneutral lakes (Almer et al. 1974) do not constitute solid evidence in the absence of preacidification data for the same lakes. In cultures of *Selenastrum*, phosphate concentration increased following acidification of the medium (Weiss 1970).

This study was designed to examine the effects of pH shock, of the magnitude observed in acidifying lakes, on phosphorus concentrations and kinetics of uptake by planktonic microorganisms. Two lakes were selected for investigation: one acidifying and the other circumneutral. The comparative approach was selected to determine whether microbial populations in a lake in incipient stages of acidification and in a neutral lake differ in their responses to pH shock. The time scale selected

[1]This paper was presented at the Phosphorus–Plankton Dynamics Sympqsium at the 27th Conference on Great Lakes Research, International Association for Great Lakes Research, Brock University, St. Catharines, Ont., May 1984.

for the pH shock experiments (30 min) would allow us to study effects of chemical changes in isolation from adaptation which would require a scale on the order of microbial generation times, i.e. several hours to several days.

Methods

The two lakes, Plastic (45°11′ N, 78°50′ W) and St. Nora (45°09′ N, 78°50′ W), are located on the Precambrian Shield in the Muskoka–Haliburton region of Ontario. Plastic Lake is a small (= 32.3 ha), shallow ($\bar{z}$ = 7.9 m), oligotrophic, clear-water lake considered to be very sensitive to acidification on the basis of alkalinities (OME 1982). Lake St. Nora is a much larger (261.8 ha), deeper ($\bar{z}$ = 17.5 m), mesotrophic lake, classified as moderately sensitive to acidification (OME 1982).

Lake water samples for experiments were collected every 4–6 wk from the offshore region. Water was collected at 1-m intervals in the epilimnion to a maximum depth of 10 m with a 5-L capacity Van Dorn sampler, and the samples were pooled in a polycarbonate carboy. Samples were only collected to 5 m under ice cover. Experiments were started within 6 h of sample collection, and the carboys were kept at ambient lake water temperature and low light intensity in the meantime.

All glassware and containers were hand-washed in dilute sulphuric acid before use and rinsed in distilled water which had been deionised by passage through an Ultrapure deioniser column (Sargent-Welch). All filters were washed before use by soaking overnight in batches of about 20 in 500 mL of dilute (about 250 m*M*) sulphuric acid. Unwashed Sartorius cellulose acetate filters, 47-mm diameter, 0.45-μm pore size, contain appreciable amounts of phosphate which is removed by the soaking procedure. The average phosphorus content of 12 unwashed filters was 0.22 ± 0.01 μg P-PO_4 per filter (C. Ewing, Life Sciences Division, Scarborough Campus, University of Toronto, Toronto, Ont., pers. comm.).

pH was measured with a Corning 130 pH meter and a combination electrode (Canlab H 5503). The instrument was calibrated daily with pH 4.5 buffer.

Soluble reactive phosphorus (SRP) in lake water was estimated by the isobutanol extraction method of Chamberlain and Shapiro (1969). Lake water samples were filtered through washed (see above) glass fiber (GFC) filters before analysis.

Experimental Design and Methods in pH Shock Experiments

The pH of five samples of lake water was adjusted to values below ambient pH by manual titration with analar, phosphate-free sulphuric acid to span the range of pH 4.0 to ambient (sixth sample). The pH adjustment was completed in 15 min. If the target pH was overshot the sample was discarded.

Analyses for Phosphate

Within 30 min of the pH shock a portion of each of the six samples was filtered on a 0.45-μm pore size cellulose acetate filter and the filtrate analysed for phosphate by the method described earlier; these were designated as postfiltration phosphate concentrations. To investigate the contribution of particles to any changes in phosphate concentrations following pH shock, analyses were also carried out on lake water samples from which particles were removed by filtration prior to pH adjustment; this series was designated as prefiltration.

To investigate phosphate, iron, and aluminum interactions as functions of pH, iron was added as $FeCl_3$ at 75 μg Fe·L^{-1} to one series of lake water samples prior to the pH shock, and aluminum as $Al_2(SO_4)_3 \cdot 16H_2O$ was added to another series at 50 μg Al·L^{-1}.

All phosphate analyses were carried out in triplicate. To bring phosphate concentrations to analytically detectable levels, samples were spiked with 2.5 μg P·L^{-1} as K_2HPO_4 immediately before pH adjustment. To test recovery of phosphate by the isobutanol extraction method and to check for any phosphate contamination in H_2SO_4, we added 2.5 μg P·L^{-1} to deionised distilled water and adjusted the pH as in pH shock experiments. Analyses at the six pH's yielded a value of 2.47 ± 0.09 μg P·L^{-1}.

Radiophosphorus Uptake Kinetics

$^{32}PO_4$ uptake kinetics were investigated at ambient lake water temperature, at ambient pH, and in five pH-shocked lake water samples. All experiments were started within 30 min of the pH shock. The pH did not change during the experiments, which lasted 1.5–2 h. Carrier-free orthophosphoric acid (^{32}P-PO_4) from New England Nuclear was used, and the time course of uptake by particles retained on 0.45-μm pore size cellulose acetate filters (Sartorius) was measured by filtering aliquots periodically over the 1.5– to 2-h experimental period. Filters were rinsed with filtered lake water to remove any adsorbed ^{32}P-PO_4 and their radioactivity measured after addition of PCS fluor (Amersham Corp.) in a Beckman model LSR 6800 liquid scintillation counter. Size fractionations were carried out on 1- and 0.2-μm pore size Nuclepore filters. Rate constants of ^{32}P-PO_4 influx and rates of phosphate uptake were calculated following Lean and Nalewajko (1976). Rate constants, k, were estimated by linear regression analysis of the slope in plots of ln % radioactivity remaining in filtrate versus time. Radiophosphorus uptake after enrichment with 0.1–15 μg P·L^{-1} as K_2HPO_4 was investigated in time course experiments of up to 2 h in duration. Michaelis–Menten parameters were calculated from Lineweaver–Burk transformations (Riggs 1970). Michaelis–Menten experiments were carried out at ambient pH and in samples in which pH was adjusted to 4.5 after enrichment with phosphorus but before isotope addition. The Rigler bioassay (Rigler 1966) was used to calculate maximum phosphate concentrations at ambient pH. In each pH shock experiment, this bioassay value was adjusted according to the appropriate curve from Fig. 1 to calculate the maximum phosphate concentration at each experimental pH; these values were then used in calculations of rates of phosphate uptake (k, min^{-1}xP-PO_4 μg·L^{-1} = P-PO_4 μg·L^{-1}·min^{-1}).

Results

During the 15-mo study period, pH in Plastic Lake ranged from 6.2 in September 1982 to 4.8 during spring runoff in 1983. In St. Nora Lake, pH was more alkaline and constant, ranging from 6.9 to 5.6 (Table 1) during the 9-mo study period. Phosphate concentrations determined by the extraction method ranged from 0.1 to 1.71 μg P-PO_4·L^{-1} in Plastic Lake. The Rigler bioassay gave considerably lower estimates, from 0.01 to 0.10 μg P-PO_4·L^{-1}. Radiophosphorus experiments showed that, in general, phosphate turnover time was shortest (<10 min) in the May–November period and far longer (25–392 min) in winter (Table 1). Only a few phosphate analyses were carried out in St. Nora Lake, but neither these nor the phosphate turnover time differed dramatically from the Plastic Lake values (Table 1).

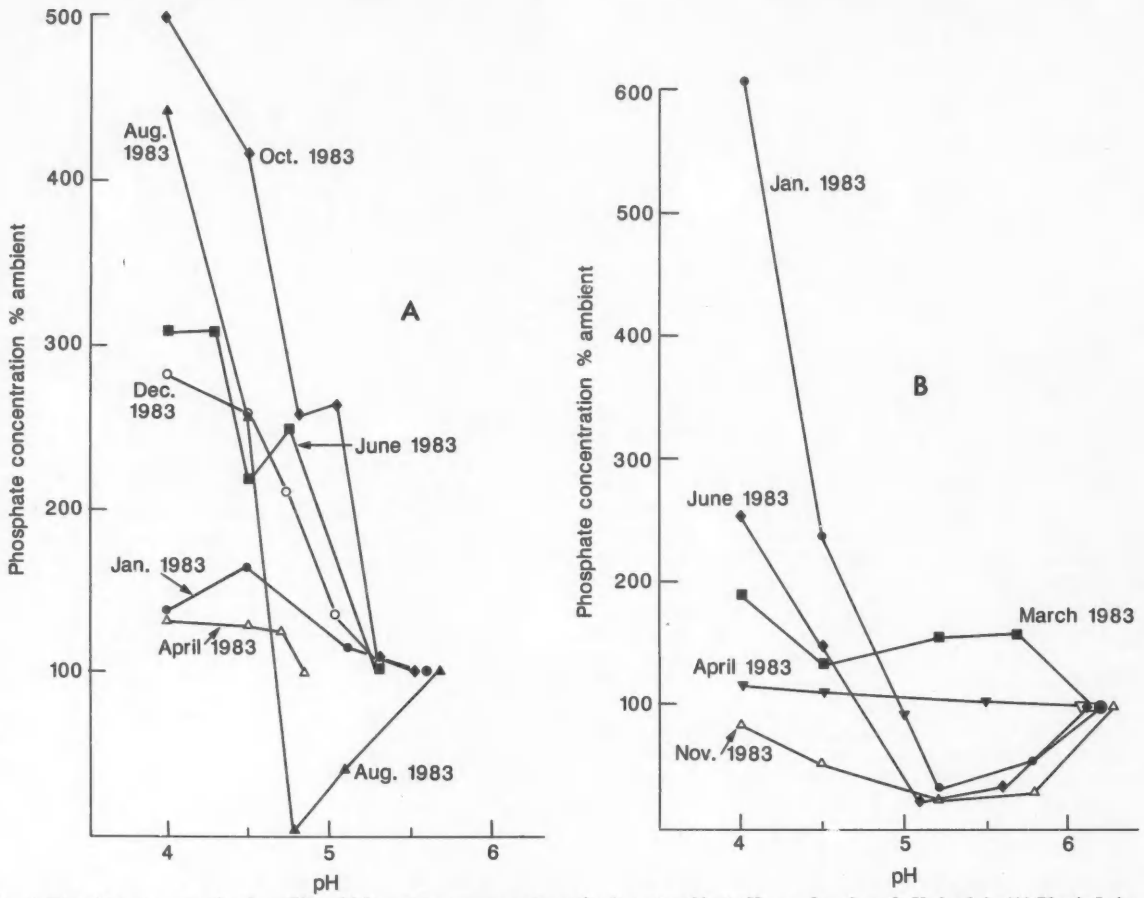

FIG. 1. Phosphate concentration in unfiltered lake water, as a percentage of values at ambient pH, as a function of pH shock in (A) Plastic Lake and (B) St. Nora Lake. Ambient pH is always the highest value for each date.

On five of six dates, an acid shock applied to Plastic Lake water (postfiltration, see Methods) resulted in increased phosphate concentrations, which reached a maximum at pH 4 (4.5 in January 1983) and a minimum in the pH 5.2–5.6 range. On one occasion (August 1983), phosphate concentration decreased on acidification to a minimum at pH 4.8 before an increase to a maximum at pH 4.0 took place. In St. Nora Lake, phosphate concentrations increased in three of five pH shock experiments to a minimum in the pH 5.1–5.8 range before increasing to a maximum at pH 4.0. In April 1983, phosphate concentrations showed little change as a function of pH. In March 1983, a small increase occurred on acidification and the maximum was reached at pH 4.0.

If particles were filtered out before the acid pH shock (prefiltration, see Methods), phosphate concentrations also changed as a function of pH, but the amplitude of the change was less extreme and phosphate minima were shifted to a lower pH (Fig. 2). Addition of iron to lake water samples resulted in a distinct phosphate concentration minimum at pH 5.0, while on addition of aluminum the minimum shifted to pH 5.9 (Fig. 3).

In pH shock experiments in Plastic Lake, rate constants (k) of ^{32}P-PO_4 uptake were highest at ambient pH and decreased with decreasing pH on 8 of 11 occasions. A decrease in k represents an increase in phosphate turnover time. For example, on 21 October 1982, phosphate turnover time ($1/k$) was 10.2 min at ambient pH (5.6), but increased about 10 times at pH 5.3 and about 100 times at pH 4.8. The ^{32}P uptake curves changed from Type 2 (Lean and Nalewajko 1979) at pH 5.6 and 5.3 to Type 1 at pH 4.8. On three occasions (31 March, 31 August, and 9 December 1983), pH shock had no effect on rate constants. In St. Nora Lake, rate constants of ^{32}P-PO_4 uptake decreased on pH shock in five of six experiments.

In all experiments in which rate constants decreased on pH shock, the decrease was a linear function of decreasing experimental pH (Fig. 4). An analysis of covariance showed that the slopes of the curves in Plastic Lake and St. Nora Lake were significantly different ($P < 0.001$).

Phosphate uptake in phosphate enrichment experiments conformed to Michaelis–Menten kinetics at ambient pH, and also at pH 4.5, but in the latter case only if ambient phosphate concentrations were not corrected for the expected increase (Fig. 1) on pH shock. The predominant response to acid pH shock was a depression of V_{max} and an increase in k_s. V_{max} was lower at pH 4.5 than at ambient pH in five of six experiments in Plastic Lake and in all five experiments in St. Nora Lake; k_s was higher

TABLE 1. Seasonal pH, phosphate concentration in filtered lake water, and phosphate turnover time in Plastic and St. Nora lakes in the epilimnion when present (e) or in 0- to 5-m integrated samples. — indicates no data.

Date	pH	Bioassay $P\text{-}PO_4$ ($\mu g \cdot L^{-1}$)	Extracted $P\text{-}PO_4$ ($\mu g \cdot L^{-1}$)	$^{32}P\text{-}PO_4$ turnover time (min)
Plastic Lake				
e 23 Sept. 1982	6.2	—	—	16.6
e 21 Oct.	5.6	0.03	—	10.1
18 Nov.	5.6	0.01	0.59	8.3
21 Jan. 1983	5.6	0.01	0.59	228.0
18 Mar.	5.7	0.05	0.1	25.3
31 Mar.	5.2	0.05	0.1	54.8
26 Apr.	4.8	0.05	0.31	54.0
e 17 May	4.8	0.01	0.41	8.8
e 17 June	5.7	0.01	0.43	8.6
e 28 June	5.3	0.10	1.40	6.7
e 3 Aug.	5.4	0.03	—	6.9
e 31 Aug.	5.6	0.03	0.05	19.5
e 20 Oct.	5.6	0.01	1.71	10.2
9 Dec.	5.6	0.01	0.86	392.0
St. Nora Lake				
e 30 Sept. 1982	6.9	—	—	16.7
e 16 Oct.	6.4	—	—	43.1
25 Nov.	6.3	—	0.78	16.8
21 Jan. 1983	6.2	—	0.1	93.1
18 Mar.	6.2	—	—	41.6
31 Mar.	5.6	—	2.61	43.8
27 Apr.	6.1	—	8.97	62.0
e 31 May	6.1	—	0.14	23.6

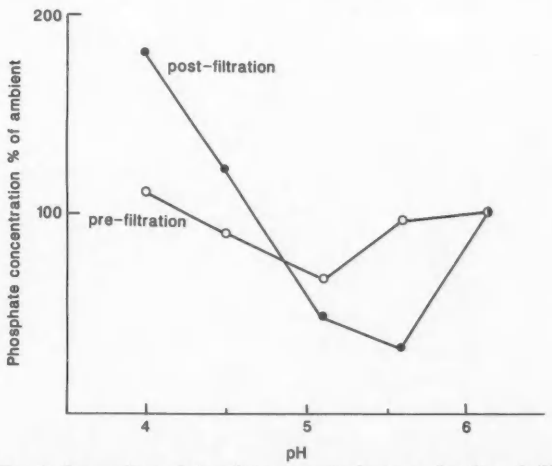

FIG. 2. Comparison of phosphate concentrations as a function of pH in whole lake water (postfiltration) and in 0.45-μm filtrates (prefiltration), St. Nora Lake, 2 June 1983. Ambient pH was 6.1.

at pH 4.5 than at ambient pH in all Plastic Lake experiments and in three of five St. Nora Lake experiments (Table 2).

We used size fractionation experiments into particles >1.0 and < 1.0 μm in an attempt to distinguish algal from bacterial uptake, respectively. In nine size fractionation experiments, at ambient pH, phosphate uptake by organisms retained on the 1-μm pore size filter ranged from 6.2 to 30.1% of total phosphate uptake in Plastic Lake (mean ± SE was 17.2 ± 7.2%). In St. Nora Lake the contribution by this size fraction was somewhat

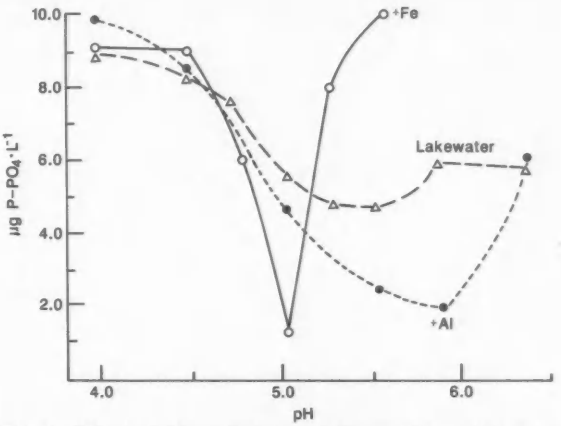

FIG. 3. Effects of 75 μg $Fe \cdot L^{-1}$ as $FeCl_3$ and 50 μg $Al \cdot L^{-1}$ as $Al_2(SO_4)_3 \cdot 16H_2O$ on the phosphate concentration–pH relationship in lake water, Plastic Lake, 9 December 1983. Analar sodium hydroxide was used to obtain pH values above ambient (pH 5.6).

higher, and much more variable, ranging from 4.3 to 65.9% (26.3 ± 25.9%). Epifluorescence microscopy showed the <1 μm fraction to be predominantly composed of bacteria, with only a few picoplankton. Conversely, the 1 μm filter retained predominantly algae, with very few attached or planktonic bacteria (O'Mahony 1985). Therefore, the use of the 1-μm pore size filter to distinguish bacterial from algal uptake appears justified in these lakes. On acid pH shock, algal phosphate uptake became a relatively more important component of total microbial uptake (Fig. 5), an indication that bacteria may be more sensitive, or respond more quickly, to pH shock. It is also possible that the increase in phosphate concentration on pH shock saturates bacterial but not algal uptake.

Discussion

The occurrence of minimal phosphate concentrations in our pH shock experiments in the pH 5.2–5.6 range leads us to speculate that further acidification of an acidifying lake with ambient pH in that range could increase phosphorus supply. Conversely, in a circumneutral lake, phosphate concentrations could decrease by as much as 75% on acidification by about 1 pH unit. Potentially, such changes in phosphate concentrations could be important to phytoplankton growth, as both Plastic Lake and St. Nora Lake are characterized by very low phosphate concentrations and by radiophosphorus turnover times indicative of phosphorus limitation in the May–October period. While a pH shock of that magnitude would not occur in 15 min, there is no reason to expect that phosphate concentrations would not change in a similar manner if the pH change occurred on a longer time scale. The occurrence of abrupt pH changes in susceptible lakes, including Plastic Lake (R. Reid, Ontario Ministry of the Environment, P.O. Box 39, Dorset, Ont. P0A 1E0, pers. comm.), is well documented. In the vicinity of Plastic and St. Nora lakes, mean pH of precipitation ranges from 3.95 to 4.22 (Dillon et al. 1979). In 1983, pH of Plastic Lake decreased from pH 5.7 to 4.8 on snowmelt in the spring.

Particles >0.45 μm appear to be the major source of phosphate released in response to pH shock, but the release of phosphorus by the organisms themselves cannot be excluded. However, some phosphorus is also released by particles <0.45 μm

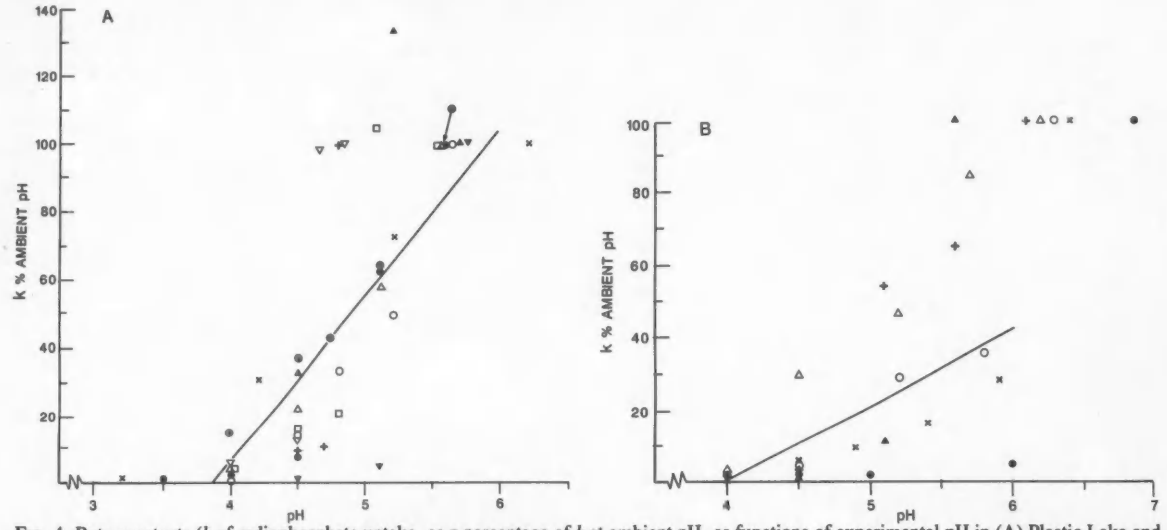

FIG. 4. Rate constants (k of radiophosphate uptake, as a percentage of k at ambient pH, as functions of experimental pH in (A) Plastic Lake and (B) St. Nora Lake. Data from experiments in which pH had no effect on radiophosphate uptake have been omitted. Values of k) at ambient pH were not used in the regression. Fig. 4A: ×, 23 Sept. 1982; ●, 21 Oct. 1982; ○, 18 Nov. 1982; Δ, 21 Jan. 1983; ▲, 18 Mar. 1983; +, 26 Apr. 1983; ∇, 17 May 1983; ▼, 1 June 1983; □, 31 Aug. 1983; ⊗, 9 Dec. 1983. Fig. 4B: ●, 30 Sept. 1982; ×, 21 Oct. 1982; ○, 25 Nov. 1982; Δ, 21 Jan. 1983; ▲, 31 Mar. 1983; + 2 June 1983. The equations of the fitted lines are as follows. Plastic Lake: k % ambient = 49.0 pH − 190.2 ($P < 0.001$); St. Nora Lake: k % ambient = 21.0 pH − 84.1 ($P < 0.01$). The slopes differ significantly ($P < 0.05$, ANCOVA).

TABLE 2. Michaelis–Menten kinetics for phosphate uptake in Plastic and St. Nora lakes. — indicates no data.

	V_{max} (μg P-PO_4·L^{-1}·h^{-1})		k_s (μg P-PO_4·L^{-1})	
Date	Ambient	pH 4.5	Ambient	pH 4.5
Plastic Lake				
18 Mar. 1983	0.80	0.62	0.24	0.42
31 Mar.	0.93	2.8	2.73	45.0
26 Apr.	3.11	0.70	0.41	1.91
17 May	3.20	0.17	0.19	1.24
31 Aug.	5.64	1.02	0.49	1.31
22 Oct.	4.22	—	2.44	—
9 Dec.	1.41	0.31	0.24	0.32
St. Nora Lake				
16 Oct. 1982	0.43	0.04	3.71	0.003
25 Nov.	1.37	—	0.77	—
21 Jan. 1983	0.37	0.18	3.27	1.31
31 Mar.	0.37	0.21	0.24	1.71
27 Apr.	1.16	0.33	0.41	2.28
2 June	1.26	0.51	0.24	1.40

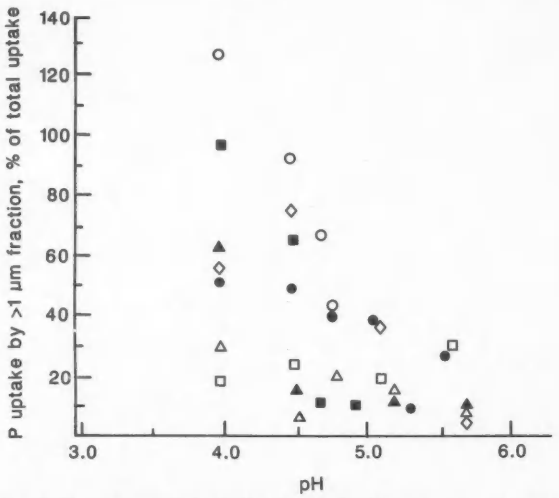

FIG. 5. Phosphorus uptake by the >1 μm fraction, as a percentage of total microbial (>0.2 μm) uptake, as a function of experimental pH, Plastic Lake. Δ, 18 Nov. 1982; □, 22 Jan. 1983; ▲, 18 Mar. 1983; ○, 26 Apr. 1983; ■, 17 May 1983; ●, 9 Dec. 1983; ◇, 1 June 1983.

(Fig. 2); these particles, and colloids, have been implicated more frequently than larger particles as sources of phosphate (Rigler 1964; Tarapchak et al. 1982). The shift in the pH at which minimal phosphate concentrations were found (Fig. 3) following aluminum and iron addition suggests that phosphate–metal interactions play an important role here. For example, the formation of iron–phosphate particles is likely to be maximal at pH 5.05, the pH of minimal solubility of iron–phosphate complexes. In contrast, aluminum–phosphate co-precipitation is likely to be maximal at pH 5.9 (Stumm and Morgan 1970). Nalewajko and Paul (1985) demonstrated maximal binding of ^{32}P-PO_4 by aluminum in Plastic Lake at pH 6.0. Minimum phosphate concentrations in both lakes occurred generally closer to pH 5.0 than 6.0, indicating that iron–phosphate interactions were dominant.

While many studies have addressed the effects of acid and molybdate concentrations, only two were concerned with the importance of exposure to acid prior to molybdate addition. Tarapchak et al. (1982) demonstrated progressive release of SRP from Lake Michigan water during exposure to hydrochloric acid, but these results are not entirely relevant to ours because the pH was probably close to 1.2. Weil-Malherbe and Green (1951) investigated the susceptibility of dissolved organic

compounds to acid molybdate hydrolysis. In their experiments, pH optima were compound specific, and only some compounds showed enhanced hydrolysis at the acid end of the pH scale. At pH 4.0, hydrolysis was minimal, usually 10%.

An increase in phosphate concentration would result in a decrease of rate constants of $^{32}P\text{-}PO_4$ uptake as a consequence of isotope dilution. The increased H^+ concentration would also decrease phosphate uptake in susceptible microorganisms. The increase in phosphate concentrations following pH shock below ambient pH is more consistent and larger in Plastic (Fig. 1A) then in St. Nora Lake (Fig. 1B), suggesting that this is the more important factor in the observed steeper decline of rate constants (Fig. 4A) in this lake compared with St. Nora Lake (Fig. 4B).

Not all of the phosphate released upon pH shock is available for microbial uptake, at least not at pH 4.5. This conclusion arises from the phosphorus enrichment experiments at pH 4.5, where kinetics obey the Michaelis–Menten equation if added plus ambient pH phosphate concentrations are used in the calculations, but not if a correction is applied to include the additional phosphate released as a result of the pH shock.

Our results on the short-term response to acid pH shock need not be an indication of long-term responses where species selection for acid and perhaps metal tolerant strains occur, and where adaptation no doubt takes place. Indeed, phytoplankton species composition in Plastic Lake is characterized by a predominance of Pyrrophyta (O'Mahony 1985) which have been documented to increase on lake acidification (Yan and Stokes 1978; Dillon et al. 1979, 1984; Yan 1979). In contrast, Chrysophyta and Bacillariophyta are the dominants on a biovolume basis in St. Nora Lake (O'Mahony 1985). However, not phytoplankton but bacteria play the major role in phosphate uptake in Plastic Lake, in contrast with St. Nora Lake where uptake by phytoplankton, although more variable than in Plastic Lake, was relatively larger. The greater sensitivity of phosphate uptake to pH shock in Plastic Lake may therefore be attributed, at least partly, to the predominance of bacterial over algal uptake. Phosphate uptake in bacteria appeared to be more susceptible than algal uptake to pH shock, suggesting that the transport mechanisms for phosphate uptake may differ in algae and bacteria. The question remains as to why bacterial uptake of phosphate predominates in Plastic Lake, and whether this is a peculiar feature of Plastic Lake or a general feature of acidifying lakes.

Some recent studies have in fact suggested that bacteria may outcompete phytoplankton at ambient phosphate levels in lakes (Paerl and Lean 1976; Currie and Kalff 1984). According to the latter, bacterial uptake of orthophosphate accounts for 97–100% of the total community uptake in Lake Memphremagog. In Plastic Lake, uptake by bacteria ranged from 69.9 to 93.8% and in St. Nora Lake from 34.1 to 97.7% of total microbial phosphate uptake.

There is no concensus on the impact of acidification on bacteria in lakes. For example, Grahn et al. (1974) suggested decreased microbial activity as an explanation of detrital accumulation in acidic lakes. This hypothesis was substantiated to some extent by Rao et al. (1984). However, direct counts revealed no changes in the abundance of planktonic bacteria on lake acidification (Traaen 1980; Kelly et al. 1984); no changes in bacterial activity were detected by Gahnstrom et al. (1980), or by Schindler and Turner (1982), and Hoeniger (1985) did not find any significant difference in cellulose breakdown rates in lakes with epilimnetic pH in the 5.5–6.9 range.

Conclusions

Short-term pH shock experiments may provide a novel approach to investigations of response of microorganisms to chemical changes that accompany acidification. Although our experiments were not designed to study long-term effects of acidification on lake water planktonic communities, our experimental design is realistic and relevant, in view of the low pH of precipitation in some areas (Dillon et al. 1979) and the occurrence of abrupt pH changes in susceptible lakes. Our data suggest that following acidification a drastic, immediate decrease occurs in radiophosphorus exchange between planktonic microorganisms and the dissolved phosphate pool. We were not able to ascertain how much of the decrease is simply due to an increase in phosphate concentrations (isotopic dilution) and how much to H^+ toxicity, but results of the phosphate enrichment experiments suggest that a large proportion of the extra phosphate is not taken up by microorganisms. We did not attempt to measure phosphate uptake rates directly because, even after the increase in concentrations and pH shock, these would have been of doubtful accuracy at such low phosphate concentration. However, from the $^{32}P\text{-}PO_4$ uptake kinetics and the Michaelis–Menten experiments, we can conclude that microbial phosphate uptake rates would be severely depressed following an acid pH shock.

Acknowledgements

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Growth Rate Responses of Lotic Periphytic Diatoms to Experimental Phosphorus Enrichment: The Influence of Temperature and Light[1,2]

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Bothwell, M. L. 1988. Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. Can. J. Fish Aquat. Sci. 45: 261–270.

Phosphate enrichment experiments were conducted year-round at the experimental troughs research apparatus (EXTRA) on the South Thompson River in British Columbia to determine the relationship between external concentration of orthophosphate and the growth rates of lotic periphytic diatom communities. Growth rate saturation always occurred at a phosphate concentration of approximately 0.3–0.6 μg P·L^{-1}. The maximum growth rate ($\mu_{max\text{-}P}$) with phosphorus enrichment varied seasonally with temperature. The relative specific growth rates (μ:$\mu_{max\text{-}P}$) as a function of external phosphate were constant. Seasonal changes in solar insolation (PAR) had no effect on the autotrophic community growth rates in unamended river water. Temperature exerted the most dominant influence on phosphorus-replete growth rates.

Afin de déterminer la relation entre les concentrations externes d'orthophosphate et le taux de croissance des communautés lotiques de diatomées (périphyton), on a mené des expériences d'enrichissement au phosphate dans les bacs expérimentaux de recherche (EXTRA) situés sur la rivière South Thompson (Colombie-Britannique). On a obtenu un plafonnement constant des taux de croissance à une concentration de phosphate allant approximativement de 0,3 à 0,6 μg·L^{-1} de P. Le taux de croissance maximum ($\mu_{max\text{-}P}$) par suite d'un enrichissement au phosphore a varié en fonction de la température saisonnière tandis que les taux relatifs de croissance selon l'espèce (μ:$\mu_{max\text{-}P}$) par rapport à la concentration de phosphate externe étaient constants. Les variations saisonnières de l'ensoleillement (PAR) n'ont eu aucune incidence sur les taux de croissance de la communauté d'autotrophes présente dans les eaux fluviables non amendées. La température est le facteur qui a le plus influé sur les taux de croissance en présence d'une surabondance de phosphore.

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On the basis of a detailed comparison of the growth rates and the physiological status of periphyton communities with respect to phosphorus deficiency in three British Columbia rivers, I concluded that at in situ soluble reactive phosphorus (SRP) concentrations of 3–4 μg·L^{-1}, periphyton growth rates were no longer limited by the availability of this primary nutrient (Bothwell 1985). Although it has long been established that the growth rates of many unicellular planktonic algae saturate under steady-state conditions at very low phosphorus concentrations, <1 μg·L^{-1} (Fuhs 1969; Rhee 1973; Brown and Button 1979), the dissolved phosphorus levels normally observed to stimulate excessive algal accumulations in rivers and streams are at least one order of magnitude higher than this (MacKenthun 1968; Wong and Clark 1976; Horner et al. 1983). Phosphorus concentrations inferred to be growth rate saturating in the Thompson River are also well below the 10–30 μg·L^{-1} levels used to enhance lotic algal production experimentally (Stockner and Shortreed 1978; Peterson et al. 1983, 1985; Perrin et al. 1987).

The discrepancy in the river literature regarding the levels of phosphorus enrichment needed to produce dense algal accumulations must be partly due to the difference between concentrations required to saturate cellular growth rates and those levels needed to maximize growth of an entire benthic mat. To answer this two-part question, a series of direct enrichment trials were conducted at the experimental troughs research apparatus (EXTRA) in Chase, British Columbia. This paper describes experiments to define the specific growth rate responses of periphytic diatoms to a range of known external orthophosphate concentrations. These trials were conducted year-round to assess how seasonal changes in solar radiation and temperature affect community growth rate responses to phosphorus enrichment. The results of other experiments describing the relationship of maximum sustainable areal algal biomass in response to phosphorus enrichment will be presented in a later paper.

Materials and Methods

Experimental Troughs Research Apparatus (EXTRA)

EXTRA is located on the South Thompson River near the outlet of Little Shuswap Lake in British Columbia (Fig. 1). Twelve parallel Plexiglas troughs, each 2 m long by 19 cm wide, were continuously fed fresh river water from a constant-

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[2]This is the second paper in a series from the experimental troughs research apparatus (EXTRA).

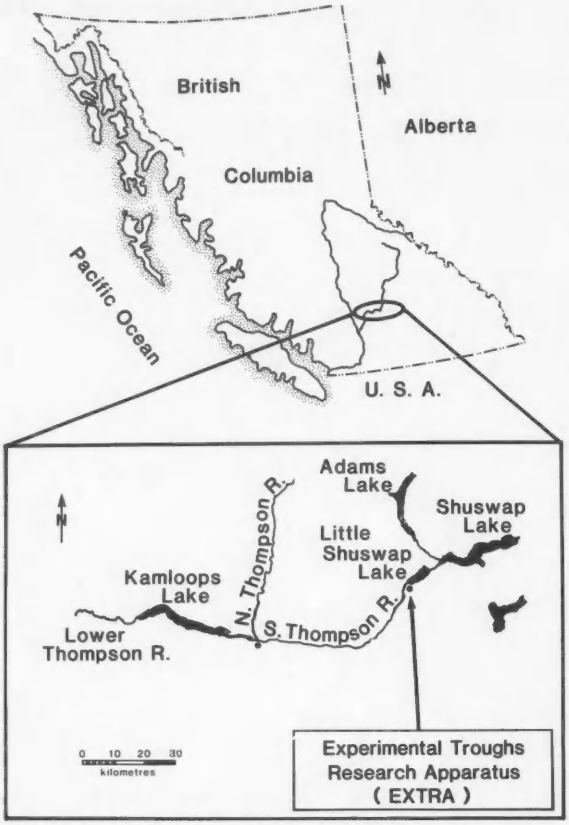

FIG. 1. Location of the experimental troughs research apparatus (EXTRA) on the bank of the South Thompson River near the outlet of Little Shuswap Lake in south central British Columbia.

pressure headtank (Fig. 2A). During these trials, flow to each trough was set at 50 $L \cdot min^{-1}$. This resulted in a water depth and velocity in the troughs of approximately 1 cm and 50 $cm \cdot s^{-1}$, respectively. Sheets of open-cell styrofoam-DB (Snow Foam Inc., El Monte, California) lined the bottom of the troughs and served as a substratum for microbial colonization (Fig. 2B). Stock solutions of K_2HPO_4 were continuously metered into the enriched troughs using high-precision piston pumps (RH type, Fluid Metering Inc., New York, NY). The phosphate solutions were injected into 20-L chambers where thorough mixing with the river water occurred prior to overflow into the troughs. This procedure assured continuous, nonpulsed phosphorus addition. Phosphate concentrations in the troughs were computed from the known dilutions of the standard enrichment solutions.

The troughs were covered with UV-opaque Plexiglas (UFI type: less than 0.5% transmission at wavelengths <360 nm) to prevent natural UV-B radiation from affecting algal growth in shallow water.

Growth Rate Experiments

Ten growth rate versus phosphorus concentration experiments were run during 1984–85: 1–27 April, 7–30 May, 11 June–6 July, 6–27 August, 22 October–7 November, 12 November–5 December, 7–25 January, 29 January–25 February, 4 April–3 May, and 27 May–24 June. Normally, seven troughs were used in these trials: one control, with no additional phosphorus and six levels of constant phosphorus enrichment: 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 $\mu g\ P \cdot L^{-1}$ as PO_4^{3-}. Three additional phosphorus enrichment trials incorporating only a control trough and a single high level of phosphorus addition (5.0 $\mu g\ P \cdot L^{-1}$) were also run. The remaining troughs were used for other types of experiments to be described elsewhere.

Each experiment began with the placement of fresh styrofoam sheets on the bottoms of the troughs and the simultaneous commencement of river water flow and phosphorus addition. The time-course of algal biomass accrual was followed using Chl *a*. Quadruplicate cores (5.0 cm^2) of the styrofoam were removed with a corkborer from all the troughs at 2- to 3-d intervals (Fig. 2C, 2D, 2E). Using this experimental trough design and sampling procedure, Chl *a* measurements on individual cores have a coefficient of variation of approximately 11% (Bothwell 1983). The replicate cores from each trough on each sampling date were immediately extracted in 90% acetone using a Polytron grinder. The extracts were combined for a single fluorometric Chl *a* determination (Holm-Hansen et al. 1965). Growth rates were computed by a least-squares fit of the time-course chlorophyll accrual data to the equation

$$y = a \cdot e^{kt}$$

where *y* is the Chl *a* concentration (milligrams per square metre) at day *t*, *a* is the initial Chl *a* concentration, and *k* is the specific net growth rate. Values of *k*, divided by 0.693, give specific growth rate (μ) expressed as divisions per day. To limit the potential influence of algal settlement (immigration) rate on the calculated growth rates (Bothwell 1983; Bothwell and Jasper 1983), Chl *a* data from the first 5–8 d were omitted. Invertebrates visible to the unaided eye were removed from the troughs with needlenose forceps each sampling date to limit the influence of grazing pressure. Experiments lasted 16–31 d and were terminated when the rate of biomass increase began to decline. The maximum growth rate attained with phosphorus enrichment (phosphorus-unlimited growth rate, here designated as $\mu_{max\text{-}P}$) during each experiment was determined by a least-squares fit to an Eadie–Hofstee transformation of the growth rate versus phosphorus concentration data (Dowd and Riggs 1965). In the three experiments with only a control trough and a single high phosphorus concentration, $\mu_{max\text{-}P}$ was assumed to be the growth rate in the enriched trough. Relative specific growth rates ($\mu : \mu_{max\text{-}P}$) were computed for the control and all levels of phosphorus enrichment in each experiment.

Alkaline Phosphatase Activity (APA)

Near the end of each growth rate experiment, periphyton were harvested from the troughs, suspended in filtered river water, and used for APA and Chl *a* analyses as well as taxonomic examination. Before subsampling, the periphyton suspensions were vigorously agitated to disrupt algal clumps and then filtered through a 550-μm-mesh Nitex net. APA determinations on triplicate 8-mL subsamples were done using the method of Pettersson (1980). Incubations were conducted for 30–60 min with 100μM 4-methylumbelliferyl phosphate (MUP) at 35°C. Reaction mixtures were buffered with Tris at pH 9.5 and had added calcium, 500 μM final concentration. The hydrolysis product methylumbelliferyl (MU) was measured fluorometrically. Total APA was corrected for dissolved APA in membrane-filtered (Millipore HA) river water. The particulate APA values were normalized to algal biomass using Chl *a*. Chlorophyll subsamples filtered onto Whatman GF/F filters were

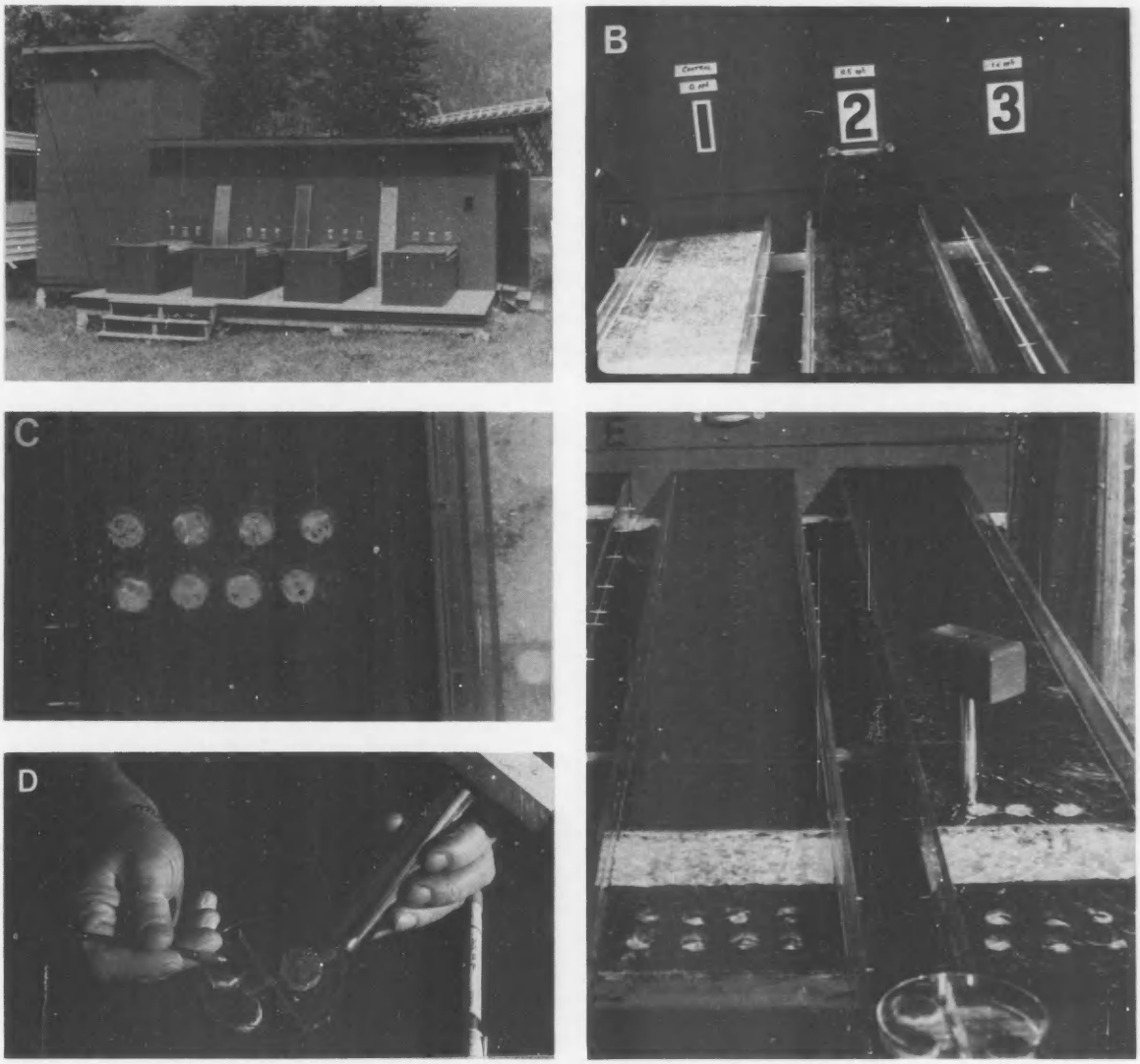

FIG. 2. (A) Front view of EXTRA showing 12 troughs, three on each of four platforms; (B) three troughs after 21 d of periphyton accumulation; (C) closeup showing holes remaining following removal of styrofoam plugs for chlorophyll analysis; (D) styrofoam plugs removed from the corkborer for chlorophyll analysis; (E) two troughs during a trial showing holes resulting from chlorophyll sampling and a section of the styrofoam removed for physiological measurements of the adhering periphyton community.

extracted in 90% acetone with a Polytron grinder and determined fluorometrically with a Turner Designs fluorometer.

^{32}P Radiobioassays

During four of the experiments, phosphate uptake kinetics by the periphyton suspensions from one of the enriched troughs were used as a bioassay of the orthophosphate content of the South Thompson River water (Rigler 1966). Natural levels of PO_4^{3-} (S_n) were augmented with a standard KH_2PO_4 solution to yield final concentrations of S_n plus (A) 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 μg P·L^{-1}. Incubations with carrier-free $^{32}PO_4^{3-}$ were carried out in room light within 1°C of ambient river temperatures. At geometrically increasing time intervals from 1 to 30 min, 10-mL subsamples were withdrawn and filtered through 0.45-μm Millipore filters. Filter-retained and total activities were counted in Aquasol-2. Uptake rate constants (k) were calculated from the slopes of the fraction of the total activity retained versus time. Initial uptake velocities (V), calculated as the product of k and the total substrate level ($S_n + A$), were plotted against $S_n + A$. As suggested by Rigler (1966), uptake velocities were recalculated assuming progressively lower values of S_n until V versus $S_n + A$ curves approximated Michaelis–Menten kinetics. This value of S_n was taken as the upper limit for PO_4^{3-} in the river water.

Algal Taxonomy

Subsamples of the periphyton suspensions fixed with Lugol's

TABLE 1. Mean values of the physical and nutrient water chemistry parameters during each of 13 experiments at EXTRA in 1984–85. NA = not available.

Experiment dates	Temperature (°C)	PAR ($E \cdot m^{-2} \cdot d^{-1}$)	SRP ($\mu g\ P \cdot L^{-1}$)	PO_4^{3-a} ($\mu g\ P \cdot L^{-1}$)	SiO_2 ($mg \cdot L^{-1}$)	$NO_3^- + NO_2^-$ ($\mu g\ N \cdot L^{-1}$)	NH_4^+ ($\mu g\ N \cdot L^{-1}$)
1984							
9 Feb. – 7 Mar.	1.8	NA	NA	NA	NA	NA	NA
1–27 Apr.	6.4	25.0	1.8	NA	6.1	62	<2
7–30 May	9.1	29.5	2.0	NA	6.3	71	<2
11 June – 6 July	12.9	39.7	1.3	0.1	6.3	77	<2
6–27 Aug.	17.9	34.6	1.9	0.1	5.4	15	<2
10 Sept. – 1 Oct.	14.3	19.3	2.1	NA	5.3	17	<2
22 Oct. – 7 Nov.	7.4	7.49	3.0	0.5	5.3	19	<2
12 Nov. – 15 Dec.	4.3	4.16	1.2	0.5	5.8	67	4
1985							
7–25 Jan.	0.80	3.74	1.9	NA	5.9	80	7
29 Jan. – 25 Feb.	0.90	9.77	1.3	NA	5.9	76	NA
28 Feb. – 25 Mar.	1.9	18.7	1.4	NA	5.8	74	5
4 Apr. – 3 May	5.4	28.3	5.4	NA	5.7	60	4
27 May – 24 June	10.7	38.6	3.7	NA	6.0	70	<2

[a]Estimated upper limit for true orthophosphate from ^{32}P radiobioassay.

solution were used for algal enumeration. Quantitative cell counts were made at 500× magnification in Utermöhl chambers. Only cells containing cytoplasm were enumerated. A minimum of 100 individuals of the dominant species and at least 300 cells in total were counted.

Water Chemistry and Physical Measurements

Triplicate samples for water chemistry were taken from the control trough at weekly intervals. All samples were filtered immediately through prewashed 0.45-μm Sartorius membrane filters. Those for SRP were preserved with chloroform. Samples for soluble reactive silicate, ammonium-N and nitrate + nitrite-N were refrigerated until analysis. All analyses were conducted by the Pacific and Yukon Regional Water Quality Branch Laboratory, Department of Environment, North Vancouver, using procedures outlined in their manual (Environment Canada 1979). During initial experiments, SRP samples were collected from the enriched troughs to check calculated additions. Because of the limited sensitivity of the analytical method for dissolved phosphorus, only the higher levels of phosphorus additions (i.e. 2.0 and 5.0 $\mu g \cdot L^{-1}$) could be accurately verified.

Water temperature was measured daily at 0900–1000 with a mercury thermometer. The mean observed temperature during each experiment was used in correlation analyses. Hourly integrals of photosynthetically active radiation (PAR) were recorded during all but one of the experiments using a LiCor (Lincoln, Nebraska) integrating light meter (LI-550B) and a quantum sensor (LI-190SB). Mean daily PAR values (Einsteins per square metre per day) were calculated for each trial period.

Results

Physical Data and Water Chemistry

The mean water temperatures during the experiments ranged from 0.8 to 17.9°C (Table 1). This seasonal variation of 17 degrees provided an opportunity to see how temperature and phosphorus interact to control growth rate. Similarly, seasonal changes in PAR resulted in mean daily values during experiments ranging from 3.74 in January to 39.7 $E \cdot m^{-2} \cdot d^{-1}$ in July (Table 1). The thermal inertia of Shuswap Lake caused vernal increases and autumnal declines in river water temperatures to lag behind changes in solar radiation. Hence, water temperature and PAR are more loosely correlated than would be otherwise expected ($r = 0.681$, $0.01 < P < 0.05$).

SRP values in the South Thompson River in 1984–85 ranged from 1.2 to 5.4 $\mu g \cdot L^{-1}$ and showed no seasonality (Table 1). Although low, these values are higher than those observed in this river in 1980–81 (mean value 0.7 $\mu g \cdot L^{-1}$; Bothwell 1985). It is not known whether this difference reflects real interannual variability or the fact that EXTRA is located 65 km upstream of the site used in the earlier studies. This uncertainty is less important than the fact that all conventional SRP procedures potentially overestimate true PO_4^{3-} (Rigler 1968; Stainton 1980). Rigler radiobioassays showed that the maximum true orthophosphate concentration in the unamended river water was 0.1 $\mu g\ P \cdot L^{-1}$ during summer (Table 1). During the late fall and early winter the upper estimate of orthophosphate increased to 0.5 $\mu g \cdot L^{-1}$. Although these bioassay estimates of PO_4^{3-} are somewhat subjective, they nevertheless provide some evidence for seasonal changes in ambient levels of PO_4^{3-} in the river.

Nitrate + nitrite levels in the winter and spring were around 60–80 $\mu g\ N \cdot L^{-1}$ but declined to less than 20 $\mu g\ N \cdot L^{-1}$ during summer and early fall (Table 1). With ammonium often below detection (<2 $\mu g\ N \cdot L^{-1}$) the combined available inorganic nitrogen was low enough during the three experiments conducted during the summer and early fall to potentially limit the growth rates of some algal species (Eppley 1977). Reactive silicate was always high, 5–6 mg $SiO_2 \cdot L^{-1}$ (Table 1), and not likely to be a factor limiting diatom production (Kilham 1971; Eppley 1977).

Algal Taxonomy

The algal periphyton communities after 2–4 wk of accumulation and growth were always dominated by diatoms. Six taxa (*Tabellaria fenestrata*, *Fragilaria crotonensis*, *F. vaucheriae*, *Diatoma tenue*, *Hannaea arcus*, and *Synedra* spp.) were consistently present, each constituting >5% of the total cell numbers throughout most of the year. Recognizable seasonal shifts occurred in the relative importance of some species. In the control, *F. crotonensis* became abundant during the spring

TABLE 2. Periphyton growth kinetic parameters during each of 13 experiments at EXTRA during 1984–85. μ = control growth rate; $\mu_{max\text{-}P}$ = phosphorus-replete growth rate; $\mu{:}\mu_{max\text{-}P}$ = relative specific growth rate of the control. Also shown are the chlorophyll-normalized, particulate alkaline phosphatase activity (APA) for the control trough during each trial and the dominant species present in the control (percent by volume). NA = not available.

Experiment dates	Growth kinetic parameters				Major algal species
	μ (div·d^{-1})	$\mu_{max\text{-}P}$ (div·d^{-1})	$\mu{:}\mu_{max\text{-}P}$	APA (nmol·h^{-1}·µg Chl a^{-1})	
1984					
9 Feb. – 7 Mar.	0.08	0.29	0.28	NA	*Synedra* spp. (21%), *Fragilaria vaucheriae* (16%)
1–27 Apr.	0.11	0.32	0.34	302	*Fragilaria crotonensis* (46%)
7–30 May	0.14	0.43	0.33	293	NA
11 June – 6 July	0.16	0.48	0.33	296	*Fragilaria crotonensis* (65%), *Synedra* spp. (14%)
6–27 Aug.	0.39	0.68	0.57	203	*Tabellaria fenestrata* (49%), *Fragilaria crotonensis* (23%)
10 Sep. – 1 Oct.	0.32	0.65	0.49	235	*Tabellaria fenestrata* (52%), *Fragilaria crotonensis* (20%)
22 Oct. – 7 Nov.	0.20	0.34	0.59	78	*Tabellaria fenestrata* (49%), *Fragilaria crotonensis* (11%)
12 Nov. – 15 Dec.	0.26	0.34	0.76	29	NA
1985					
7–25 Jan.	0.23	0.26	0.88	70	*Tabellaria fenestrata* (55%), *Synedra* spp. (14%)
29 Jan. – 25 Feb.	0.11	0.18	0.63	106	*Tabellaria fenestrata* (37%), *Diatoma tenue* (34%)
28 Feb. – 25 Mar.	0.15	0.24	0.61	103	NA
4 Apr. – 3 May	0.10	0.26	0.38	392	*Tabellaria fenestrata* (58%), *Fragilaria vaucheriae* (13%)
27 May – 24 June	0.25	0.58	0.43	172	*Tabellaria fenestrata* (44%), *Fragilaria crotonensis* (32%)

and waned during the autumn (Table 2). *Tabellaria fenestrata* first became a dominant species in August of 1984 but remained an important contributor to algal biomass through the remainder of the study period (Table 2).

Algal Growth Rates

During all experiments and under all treatments, algal biomass increased exponentially between weeks 1 and 3. The rate of Chl *a* increase proved to be a consistent and reliable measure of the periphyton community growth rate. Chlorophyll accrual data used in the computation of growth rates during one of the experiments (1–27 April 1984) are shown in Fig. 3. This figure illustrates that statistically significant ($P < 0.01$) increases in growth rate (*F*-test comparisons of the slopes of the lines; Sokal and Rohlf 1969) occurred with addition of just 0.5 µg P·L^{-1}. In the April 1984 trial, there were no differences between the 0.5, 1.0, 2.0, and 5.0 µg P·L^{-1} treatments (Fig. 3). The lack of additional significant ($P < 0.05$) growth rate response with PO_4^{3-} additions above 0.5 µg P·L^{-1} was found in 5 of the 10 experiments. In fact, even though growth rates of the diatom communities always increased above control levels ($P < 0.05$) with addition of just 0.1 µg P·L^{-1}, levels above 0.2 µg P·L^{-1} had no additional affect on growth rate during some months of the year.

Although the augmentation in external phosphorus concentration needed to saturate the periphyton community growth rates was always low, there were large changes in the maximum growth rates attained at different times of the year (Fig. 4). Seasonally, $\mu_{max\text{-}P}$ varied from 0.18 to 0.68 div·d^{-1} (Table 2). Higher $\mu_{max\text{-}P}$ values were reached during the summer than during the winter. The maximum growth rate correlated very highly with temperature ($r = 0.941$, $n = 13$; Table 3), accounting for nearly 90% of the annual variability in $\mu_{max\text{-}P}$. Mean daily PAR during experiments also showed a significant correlation with $\mu_{max\text{-}P}$ ($r = 0.585$, $P < 0.05$), but only accounted for 34% of the observed variation.

Algal growth rates in the control trough ranged from 0.08 to 0.39 div·d^{-1} (Table 2). Seasonal changes in PAR did not correlate with the growth rates of the periphyton communities in the unamended river water (Table 3). However, annual temperature fluctuations did correlate with growth rate in the control ($r = 0.661$), explaining 44% of the observed variation.

Normalizing μ to $\mu_{max\text{-}P}$ helps to factor out the influence of physical conditions (primarily temperature) on growth rates (Goldman 1980). The normalized growth response curves for South Thompson River periphytic algae were similar at all times of the year when plotted over the full range of added phosphate from 0 to 5 µg P·L^{-1} (Fig. 5). This similarity provides dramatic documentation of the usefulness of the relative specific growth rate concept in predicting algal growth rate responses to phosphorus enrichment under varying physical conditions in situ.

Alkaline Phosphatase Activity

Chlorophyll-normalized APA values in the control showed clear seasonal trends (Fig. 6A). Maximum APA of 200–400 nmol MU·h^{-1}·µg Chl a^{-1} occurred during the spring and summer, while lower values (<100 nmol MU·h^{-1}·µg Chl a^{-1}) occurred during the late fall and winter (Table 2; Fig. 6A). APA is a well-established indicator of phosphorus deficiency in many algae (Berman 1970; Jones 1972; Healey and Hendzel 1979; Gage and Gorham 1985) and has been shown to be a reliable index of relative phosphorus limitation in periphyton in this river system (Bothwell 1985). The highest values of APA in the untreated periphyton communities corresponded to very low levels of ambient PO_4^{3-} (Rigler radiobioassay estimates were ≤0.1 µg P·L^{-1}) in July and August 1984 (Tables 1, 2). Lower APA occurred when PO_4^{3-} levels were estimated to be higher (≤0.5 µg P·L^{-1}; Tables 1, 2). While APA in the control trough showed no relationship to growth rate ($r = -0.305$; Table 3), the correlation with the relative specific growth rate throughout the year was highly significant and negative ($r = -0.864$; Table 3; Fig. 6A). APA also showed a highly significant positive correlation with light (Table 3). The negative correlation with $\mu{:}\mu_{max\text{-}P}$ confirms that Chl *a* normalized APA is a useful indicator of relative phosphorus deficiency in these periphyton communities. However, the observation that APA did not cor-

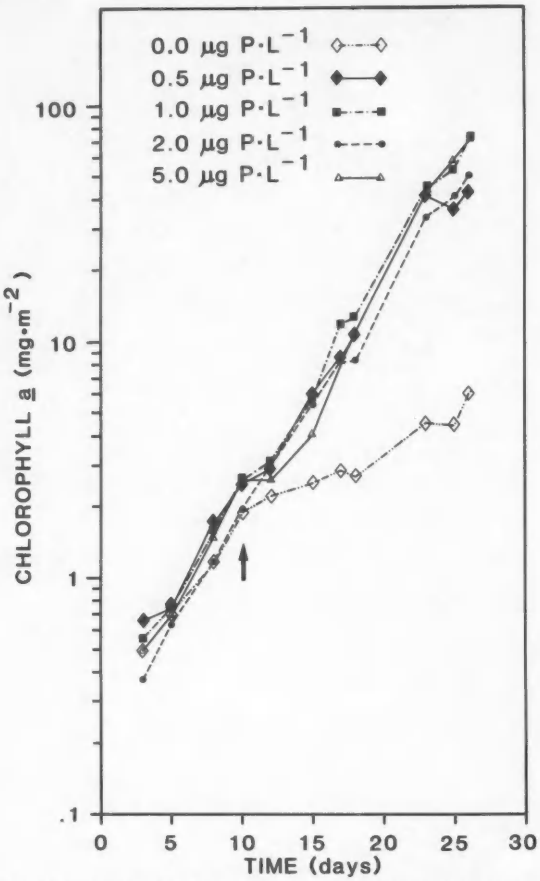

FIG. 3. Semi-log plot of time-course curves of chlorophyll accumulation during one experiment, 1–27 April 1984. Arrow depicts the break point usually separating colonization from exponential growth phase of an experiment. Data from this point to the end of the experiment were used in calculating growth rates. Highly significant ($P < 0.01$) increases in algal growth rates with additions of orthophosphate of 0.5 µg P·L⁻¹ and above are apparent. Higher phosphorus concentrations did not significantly stimulate growth rates above those found at 0.5 µg P·L⁻¹.

relate with the control µ shows that APA is not a reliable index of absolute growth rate.

Discussion

Phosphate Concentration versus Growth Rate

These experiments provide conclusive evidence that the concentrations of dissolved orthophosphate required to saturate the specific cellular growth rates of periphytic riverine diatoms are extremely low. Conservatively, saturating concentrations are well under 1 µg P·L⁻¹. It is worth emphasizing that these concentrations are near the detection limits of standard wet chemical techniques for dissolved phosphorus. The disparity between the conclusion that saturating levels of orthophosphate are well below 1 µg P·L⁻¹ and my earlier suggestion that 3–4 µg SRP·L⁻¹ is needed to approach growth rate saturation in diatom dominated rivers (Bothwell 1985) is further testimony

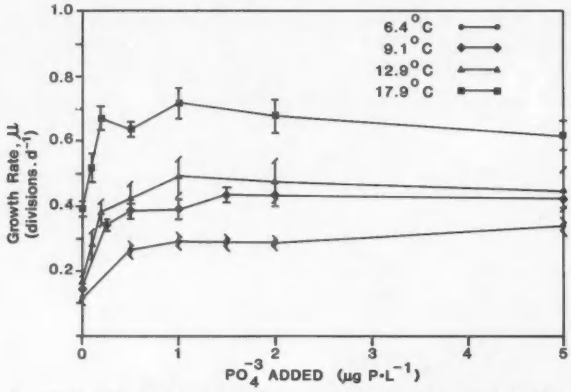

FIG. 4. Attached algal community growth rates as a function of added orthophosphate concentration at different times of the year (i.e. different temperatures). For clarity of presentation the results of only 4 of the 10 experiments are shown. Error bars are ±1 SD.

to the fact that SRP overestimates true orthophosphate in natural waters.

The futility of assessing phosphorus limitation of algal growth rates on the basis of SRP measurements alone was confirmed by three independent measures in this study. First, although SRP in unamended South Thompson River water showed no seasonal trends during the year, the APA data showed clear temporal changes in the level of phosphorus deficiency in the periphytic algae (Fig. 6A). Second, $\mu:\mu_{max\text{-}P}$ in the control trough periphyton community confirmed the same trend seen in the APA data, showing marked seasonality in phosphorus limitation in the river (Fig. 6A). Lastly, radiobioassays for true orthophosphate agreed with the other biological indicators of phosphorus availability and suggested that the concentrations of true phosphate were higher in the fall and early winter than in the summer. Hence, while SRP is an important measure of the level of biologically available phosphorus, the present data show a considerable range in phosphorus-limited growth rates in situ which are not reflected in ambient SRP measurements. Care must be taken to include other indicators when evaluating the relative degree of phosphorus deficiency in lotic systems.

The apparent similarity of response in relative specific growth rates to phosphorus enrichment at different times of the year (seen in Fig. 5) was surprising in the face of several lines of evidence indicating seasonal changes in background concentrations of phosphate in the river. Congruity would be expected only if orthophosphate levels in the river were constant. A plot of seasonal $\mu:\mu_{max\text{-}P}$ trends in response to only the lowest levels of phosphorus addition shows that there were clear temporal changes in how the relative specific growth rates of periphyton communities responded to low levels of phosphorus enrichment (Fig. 6B). In other words, the initial slopes of the response curves in Fig. 5 did vary seasonally but these changes are not evident on a phosphorus versus µ plot covering the full range of added phosphate concentrations. The varying responses to phosphate enrichment reflect seasonal changes in $\mu:\mu_{max\text{-}P}$ in the natural river communities due to very small changes in the background levels of orthophosphate in the river. When APA in the control periphyton was 200–400 nmol·h⁻¹·µg Chl a^{-1}, phosphorus additions of 0.5 µg P·L⁻¹ were needed to approach growth rate saturation. When river periphyton APA was lower, i.e. <100 nmol·h⁻¹·µg Chl a^{-1},

TABLE 3. Correlation coefficient matrix of physical and biological parameters during 13 phosphorus enrichment experiments at EXTRA during 1984–85. *Significant, $P < 0.05$; **highly significant, $P < 0.01$.

			Control		
	Temp.	$\mu_{max\text{-}P}$	μ	$\mu:\mu_{max\text{-}P}$	APA
PAR	0.681*	0.585*	0.038	−0.794**	0.726**
Temp.		0.941**	0.661*	−0.320	0.424
$\mu_{max\text{-}P}$			0.769**	−0.218	0.244
μ				0.436	−0.305
$\mu:\mu_{max\text{-}P}$					−0.864**

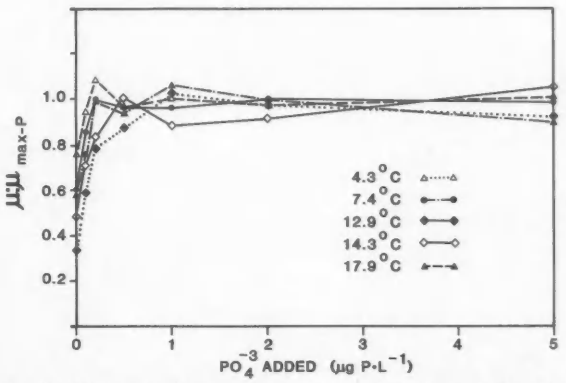

FIG. 5. Relative specific growth rates ($\mu:\mu_{max\text{-}P}$) of the attached algal communities as a function of added orthophosphate concentration at different times of the year (i.e. different temperatures). For clarity of presentation, values from only 5 of the 10 experiments are plotted.

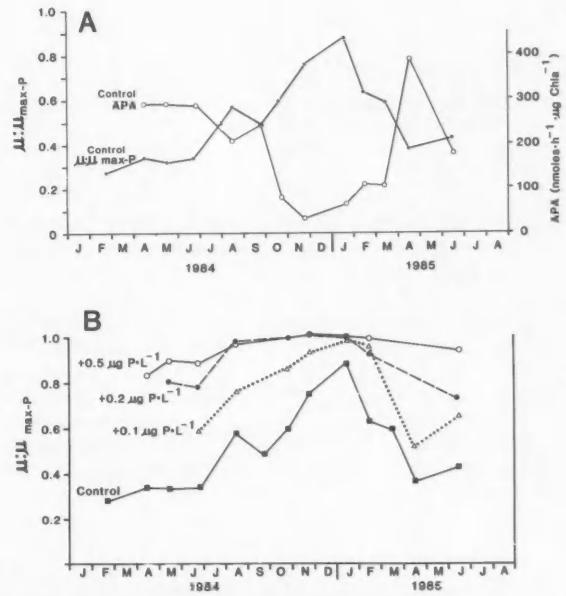
A
Control APA
Control μ:μ max-P
μ:μ max-P
APA (nmoles·h-1 μg Chla-1)
J F M A M J J A S O N D J F M A M J J A
1984
1985
B
+0.5 μg P·L-1
+0.2 μg P·L-1
+0.1 μg P·L-1
Control
μ:μ max-P
J F M A M J J A S O N D J F M A M J J A
1984
1985

FIG. 6. (A) Seasonal trends in APA and $\mu:\mu_{max\text{-}P}$ values in the control trough; (B) seasonal trends in $\mu:\mu_{max\text{-}P}$ values in the control trough and treatments with very low levels of phosphate enrichment.

additions of 0.2 or even 0.1 μg P·L^{-1} saturated growth (Fig. 6A, 6B).

The fact that the amounts of added PO_4^{3-} required to saturate growth varied in conjunction with independent measures of the ambient levels of dissolved available phosphorus in the river water suggests that the relative specific growth curves as a function of the total ambient PO_4^{3-} concentration did not change significantly seasonally. During the spring and summer, additions of 0.2–0.5 μg P·L^{-1} were needed to achieve a $\mu:\mu_{max\text{-}P}$ of 0.8–1.0 (Fig. 6B). Radiobioassays indicated a maximum PO_4^{3-} during the summer of 0.1 μg P·L^{-1}. Therefore, the total external concentration which saturated growth rate was approximately 0.3–0.6 μg P·L^{-1}. During the late fall and early winter when true background PO_4^{3-} levels were higher (a maximum of around 0.5 μg·L^{-1}), addition of only 0.1 μg·L^{-1} was needed to reach relative specific growth rates of 0.8–1.0. This sum, 0.6 μg P·L^{-1}, required for growth rate saturation is within the range of estimates from the summer period. The agreement is both remarkable and reassuring. It demonstrates that the relative specific growth rates of the diatom communities in the South Thompson River, as a function of the total external phosphate concentration, are nearly constant throughout the year and that saturation always occurs at an ambient phosphate level of around 0.3–0.6 μg P·L^{-1}.

Influence of Light and Temperature

Another important and surprising finding in this study was that seasonal changes in light did not exert an influence on algal growth rates in the control trough. The algal communities in the present study were grown in unshaded (PAR) conditions in very shallow water (approximately 1 cm deep) and protected from UV-B radiation. There was no relationship between the growth rates of natural river periphyton communities and PAR (Fig. 7D). This appears to be contrary to one of the central shibboleths of stream ecology: that algal production in temperate streams is often restricted by light availability (McIntire 1973). This tenet is supported by the frequent observation that decreased shading of streams results in increased algal biomass (Hansmann and Phinney 1973; Lyford and Gregory 1975; Murphy and Hall 1981; Sheath et al. 1986). The apparent contradiction with the present findings is partly the result of nutrient limitation. When phosphorus is not limiting, i.e. at $\mu_{max\text{-}P}$, the correlation with light is significant ($r = 0.585$, $P < 0.05$; Table 3). In the South Thompson River, phosphorus is the nutrient normally controlling algal growth rates. Seasonally, the relative degree of phosphorus deficiency varies inversely with solar radiation. PAR showed highly significant negative and positive correlations with $\mu:\mu_{max\text{-}P}$ and APA, respectively (Table 3). Temporal inverse correlation between nutrient avail-

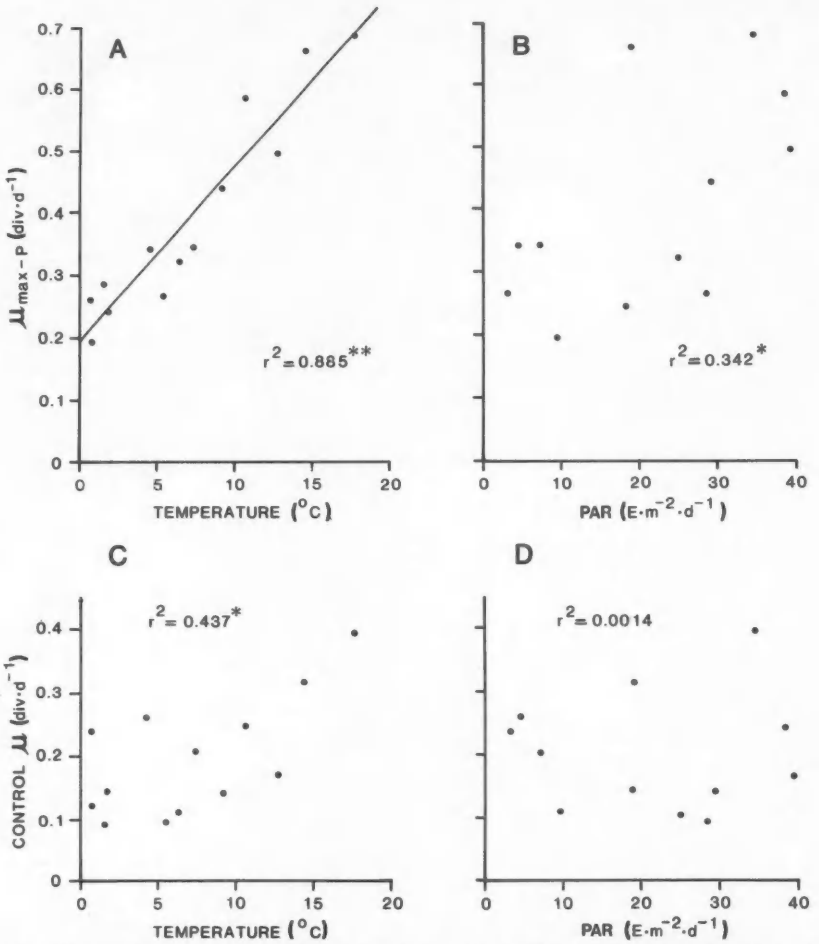

FIG. 7. Scatter diagrams of the maximum growth rate attained with phosphorus enrichment (μ_{max-P}) versus (A) temperature and versus (B) light; and the control growth rate (μ) versus (C) temperature and versus (D) light. Temperature and light data are the mean values during each experimental period for which μ and μ_{max-P} were measured. *$P < 0.05$; **$P < 0.01$.

ability and light is common in temperate, nutrient-limited aquatic ecosystems. The present results show that in non-shaded, temperate systems, diatom growth rates will not necessarily be higher during times of the year when more sunlight is available. The lesson for lotic system ecologists is simple. Conclusions about the relative importance of light on algal production in a particular stream cannot be made in the absence of specific information on the presence or absence of nutrient limitation.

Comparing the correlations of temperature with growth rates in the control trough and under conditions of unlimited phosphorus availability also points out the interaction between nutrient and physical limitation of growth (Fig. 7A, 7C). When phosphorus is not limiting, temperature exerts the dominant control over algal growth rates, explaining nearly 90% of the observed annual variation in μ_{max-P} (Fig. 7A). Under natural, seasonally varying nutrient limitation, temperature still correlates significantly with growth rate but the relationship is much weaker (Fig. 7C). The overall dominance of temperature as the physical factor controlling algal growth in these experiments may in part be due to the shallow, exposed nature of the troughs. While the conclusions reached here on the relative importance of temperature and light are valid for shallow, exposed systems, in streams with deeper water and extensive shading from shoreline vegetation, light should play a more important role than found in this study.

The correlation of μ_{max-P} with temperature ($r = 0.941$, $n = 13$) is impressive for in situ studies. It is also noteworthy that the relationship appears to be linear (Fig. 7A, 8). Theoretically, the maximum, nutrient-replete growth rate should vary as an exponential function of temperature (Eppley 1972; Goldman and Carpenter 1974). The fact that the observed μ_{max-P} was best fit as a linear function of temperature ($F[1,11] = 88.14$, $P < 0.001$; Fig. 8) suggests that at warmer temperatures, μ_{max-P} was substantially lower than the true μ_{max} because some other nutrient became limiting under phosphorus-enriched conditions. Since the nitrate levels in the summer may be low enough to limit the growth rates of some diatom species (Eppley 1977),

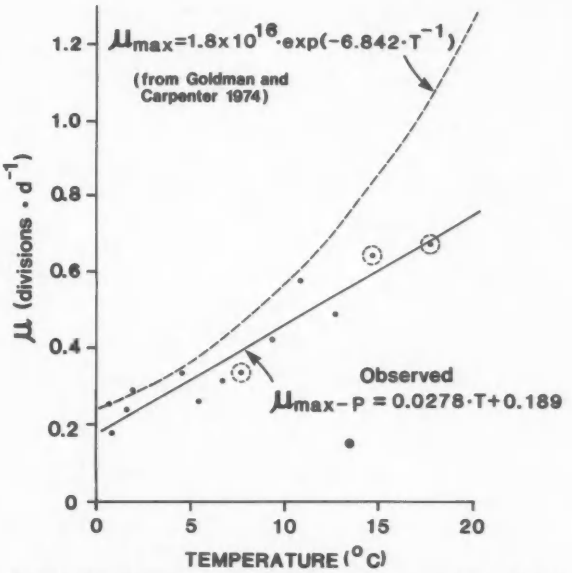

FIG. 8. Phosphorus-replete periphyton growth rates observed in 13 separate experiments versus temperature. The data are fit by least-squares to a linear function shown by the solid line. *T* in this equation is degrees centigrade. The broken line is the theoretical relationship between maximum growth rate and temperature (Goldman and Carpenter 1974). *T* in this equation is degrees Kelvin. Points circled with broken lines are values obtained when nitrate + nitrite levels were under 20 $\mu g \cdot L^{-1}$.

the coincidence of the lowest nitrate levels at some of the warmest seasonal temperatures supports the possibility of a secondary nutrient limitation (Table 1). Furthermore, direct nitrate enrichment trials now corroborate this hypothesis (M. L. Bothwell, unpubl. data).

In summary, these studies have proven that the concentrations of ambient dissolved orthophosphate required to saturate the specific cellular growth rates of periphytic diatoms in the South Thompson River are approximately 0.3–0.6 $\mu g\ P \cdot L^{-1}$. The absolute growth response to phosphorus addition varied seasonally with changes in temperature, light, and background levels of PO_4^{3-}. However, the relative growth rate of the periphyton communities as a function of total external phosphate concentration remained constant throughout the year. Seasonal changes in light intensity had no effect on the autotrophic community growth rates in unamended river water, while temperature exerted the most dominant influence on phosphorus-replete growth rates. As a final note, it is important to remember that the very low phosphorus levels which saturate the growth rates of relatively thin periphyton films do not mean that higher phosphorus concentrations will not potentially further stimulate areal periphyton biomass. The control of areal biomass by limiting nutrients is complex and the subject of a forthcoming paper (M. L. Bothwell, in prep.).

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Response of Lake Michigan Benthic Algae to in situ Enrichment with Si, N, and P[1]

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Carrick, H. J., and R. L. Lowe. 1988. Response of Lake Michigan benthic algae to in situ enrichment with Si, N, and P. Can. J. Fish. Aquat. Sci. 45: 271–279.

The possibility that benthic algae in the nearshore area of Lake Michigan might be growth limited by Si was tested using nutrient-releasing substrata. Nutrient treatments were Si, N + P, Si + N + P, and controls (CONT) and were sampled after 7, 14, and 31 d of exposure. Addition of Si alone had little stimulatory effect on algal biomass, while enrichment with Si + N + P led to the greatest increase in chlorophyll *a*, particulate Si, total biovolume, and diatom biovolume after 14 d of incubation ($P < 0.0001$). By day 31, communities on CONT and Si substrata exhibited little change in biomass and remained dominated by diatoms (98% of total biovolume), while algal biomass on both N + P and Si + N + P substrata increased more than eightfold ($P < 0.0001$) and consisted mainly of *Stigeoclonium tenue* (Chlorophyta) and *Schizothrix calcicola* (Cyanophyta). These results indicate that benthic diatoms in Lake Michigan are not currently limited by Si, but may become Si limited following enrichment with N + P.

On a vérifié la possibilité que la croissance des algues benthiques de la zone littorale du lac Michigan soit limitée par le Si, à l'aide de substrats libérateurs de bioéléments. Ces substrats, contenant du Si, du N + P et du Si + N + P ainsi que les sédiments témoins ont été échantillonnés après 7, 14 et 31 j d'exposition. L'apport de Si a eu peu d'incidence stimulante sur la biomasse algale tandis que l'enrichissement avec du Si + N + P a entraîné la plus grande augmentation de la concentration de chlorophylle-a, de Si particulaire, du biovolume total et du biovolume des diatomées après 14 j d'incubation ($P < 0{,}0001$). Après 31 j, les communautés poussant sur des substrats témoins et enrichis de Si ont montré peu de variations de la biomasse et étaient surtout constituées de diatomées (98 % du biovolume total) tandis que la biomasse algale sur les substrats enrichis de N + P et de Si + N + P a augmenté plus de 8 fois ($P < 0{,}0001$) et se composait surtout de *Stigeoclonium tenue* (Chlorophytes) et *Schizothrix calcicola* (Cyanophytes). Ces résultats révèlent que les diatomées benthiques du lac Michigan ne sont pas actuellement limitées par la concentration de Si mais peuvent le devenir par suite d'un enrichissement au N + P.

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The role nutrients play in shaping phytoplankton communities has received much attention (Kilham and Kilham 1978; Tilman 1982). Investigators have demonstrated that the availability of one to a few nutrients limits phytoplankton growth and leads to shifts in community composition (Dillon and Rigler 1974; Schindler 1977). Although less thoroughly understood, nutrients have been shown to limit benthic algal productivity (Krewer and Holm 1982). However, nutrients commonly limiting to phytoplankton (i.e. P) are often times less well correlated with benthic algal biomass (Cattaneo 1987), perhaps suggesting that nutrient supplies available to benthic algae may differ from the open water (Alexander et al. 1982; Pringle 1985). Recent work by Pringle (1987) suggests that nutrients supplied from substrata interact with nutrient supplies in the water column to mediate the response of benthic algae to enrichment.

Results from several bioassay techniques have demonstrated benthic algal growth stimulation with additions of P (Krewer and Holm 1982; Fairchild and Lowe 1984), N (Grimm and Fisher 1986), and both P and N (Stockner and Shortreed 1978; Pringle and Bowers 1984). Silicon may be another important nutrient to benthic algae, particularly because diatoms dominate most benthic algal assemblages (Stockner and Armstrong 1971; Round 1981) and dissolved Si concentrations are very low in many aquatic systems (Alexander et al. 1982; Schelske et al. 1986). While very little information exists on the role Si plays in regulating benthic algal growth and community dynamics (e.g. Hooper-Reid and Robinson 1978), Si demand by phytoplankton can serve as an index of eutrophication (Kilham 1971).

In Lake Michigan, the growth of planktonic diatoms and subsequently their rate of Si utilization has been shown to increase following P enrichment (Schelske and Stoermer 1972; Schelske et al. 1986). This phenomenon has been suggested as a

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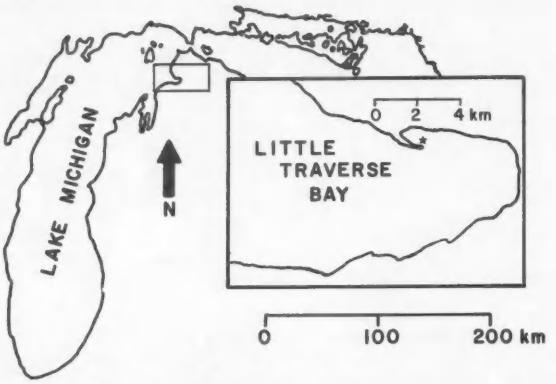

FIG. 1. Lake Michigan with Little Traverse Bay enlarged displaying the study site, indicated with a star (after Schelske and Callender 1970; Rea and Pigula 1979).

mechanism partially responsible for seasonal shifts from a diatom-dominated assemblage during spring mixing to dominance by phytoplankton without growth requirements for Si following thermal stratification (Schelske and Stoermer 1971; Fahnenstiel and Scavia 1987a). Because diatoms also dominate Lake Michigan benthic algal assemblages (Stevenson and Stoermer 1981; Kingston et al. 1983), elevated P imputs might induce similar shifts in benthic algal community composition. Thus, the present study utilized substrata which release combinations of Si, N, and P in the littoral zone of Lake Michigan in order to determine (1) whether benthic diatoms are Si limited and (2) if a shift from a diatom-dominated community to one composed of algae without Si requirements (i.e. green and blue-green algae) is observed following N + P enrichment.

Materials and Methods

Nutrient-releasing substrata were constructed as described by Fairchild and Lowe (1984) from clay flower pots (internal volume = 245 mL) that were autoclaved and soaked in deionized water for 48 h. Each substratum was filled with a hot 2% agar-deionized water solution containing one or a combination of 0.05 *M* $NaNO_3$ (N), 0.05 *M* NaH_2PO_4 (P), and 0.1 *M* Na_2SiO_3 (Si).

Nutrient Release Experiments

Release rates of each ion were determined under laboratory conditions in order to characterize the pattern and magnitude of nutrient release over a time period comparable with that of the in situ experiment. Release of NO_3-N (average release = 254 $\mu mol \cdot d^{-1}$) was higher than PO_4-P (82 $\mu mol \cdot d^{-1}$); however, both decreased through time (Fairchild et al. 1985). Release of SiO_2 was determined by filling three replicate substrata with agar and 0.1 *M* Na_2SiO_3 (Si) and three more with just agar (CONT). The Si and CONT substrata were placed in 4-L plastic containers filled with 1.5 L of deionized water. The water in each jar was changed daily for 23 d. On days 1, 2, 3, 6, 9, 12, 15, 18, 21, and 23 of the experiment, water samples were filtered through washed 0.45-μm Millipore® filters, stored in acid-washed polyethylene bottles, and refrigerated. These samples were analyzed for SiO_2 colormetrically using a Technicon II autoanalyzer (Davis and Simmons 1979).

In situ Nutrient Manipulation

Forty-eight nutrient-releasing substrata were constructed for field experiments: 12 contained Si as a nutrient addition (Si substrata), 12 had both N and P added (N + P substrata), 12 had all three nutrients added (Si + N + P substrata), and the remaining 12 served as controls, with no nutrients added (CONT substrata). On 15 July 1984, all substrata were placed approximately 0.5 m apart in a grid at a depth of 10 m off Harbor Point in Little Traverse Bay, Lake Michigan, using SCUBA (Fig. 1). Each substratum was secured into the sediments by a wooden dowel at its base. Four substrata of each treatment were gently collected after 7, 14, and 31 d in 1-gal zip-lock plastic bags by divers, taking care to include as little lake water (and suspended algae therein) as possible with each substratum.

Upon returning to the laboratory, the contents from each zip-lock bag were transferred to a 1-L wide-mouth jar. Organisms were removed from the surface of each substratum with a razor blade and toothbrush and washed into the jar with deionized water. Each sample was diluted to a known volume, from which subsamples were removed to determine concentrations of chlorophyll *a*, particulate Si (PSi), and algal biovolume (Stoermer and Kreis 1980). The remaining slurry was concentrated by settling and diatom reference slides were prepared (Patrick and Reimer 1966).

Chlorophyll subsamples were filtered (0.45-μm Millipore), sonicated, centrifuged, extracted in 90% acetone buffered with $MgCO_3$, and analyzed using a Turner 111 fluorometer. Samples were subsequently acidified and assayed a second time for phaeopigments (Strickland and Parsons 1968). Subsamples for PSi were filtered (0.40-μm Nuclepore®), transferred to plastic vials, and frozen. Samples were later subjected to NaOH extraction and acid neutralization and analyzed with an autoanalyzer for dissolved SiO_2 (Krausse et al. 1983).

Algal densities were estimated from each prepared slide by enumerating between 350 and 3000 algal cells using a Leitz Dialux® research microscope at 1250× magnification. Biovolume estimates were obtained for taxa by determining the mean cell dimensions of at least 10 randomly chosen cells. Average biovolume was calculated from the mean cell dimensions and was multiplied by cell densities to obtain the total biovolume for each taxon.

Estimates of algal biomass were analyzed using one-way analysis of variance (ANOVA), with nutrient treatments considered a fixed factor and sampling intervals treated as a blocked factor. Data were log transformed to meet the assumptions of homoscedasticity, and Student–Newman–Keuls multiple range tests ($P < 0.05$) were used to evaluate sources of significant variation (Zar 1983).

Physicochemical Analyses

Duplicate 1-L lake water samples were collected on days 1, 7, 14, and 31 of the experiment from depths of 1, 5, and 10 m using a 2-L Van Dorn bottle. Lake water samples (100 mL) were analyzed for chlorophyll, following which the filtrate was analyzed for NO_3-N, soluble reactive P (SRP), Cl^-, and SiO_2 using a Technicon II autoanalyzer (Davis and Simmons 1979). An additional 100 mL of lake water was filtered onto membrane filters to determine PSi concentrations.

Also, on days 7, 14, and 31, pH, dissolved oxygen, temperature, and conductivity were determined at 1-m intervals from the surface to depths of between 6 and 12 m using a

TABLE 1. Pooled estimates for chemical and biological parameters measured on four dates from duplicate water samples taken at three depths (1, 5, and 10 m) in Little Traverse Bay, Lake Michigan (und = undetectable).

Date (1984)	SiO_2 ($mg \cdot L^{-1}$)	Cl^- ($mg \cdot L^{-1}$)	NO_3-N ($\mu g \cdot L^{-1}$)	SRP ($\mu g \cdot L^{-1}$)	PSi ($mg \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)
15 July	0.36	8.77	182.8	und	2.14	1.11
22 July	0.41	8.77	193.1	1.00	2.81	1.65
29 July	0.35	8.62	199.4	1.20	1.55	1.15
15 August	0.37	8.23	198.0	und	1.24	0.79
Mean	0.38	8.59	193.3	1.10	1.94	1.18
SE	0.01	0.13	3.7	0.10	0.35	0.19

Hydrolab®. Water transparency was measured with a white and black Secchi disk (diameter = 20 cm).

Results

Silica (SiO_2) liberation from Si and CONT substrata was characterized by high initial release, followed by relatively constant release. The magnitude of SiO_2 release from Si substrata (254 $\mu mol \cdot d^{-1}$) was more than 25-fold higher than that released from CONT substrata (10 $\mu mol \cdot d^{-1}$).

Physicochemical Conditions of Lake Michigan Water

Concentrations of SiO_2, Cl^-, NO_3-N, and SRP showed little temporal or spatial variability (Table 1). Levels measured were similar to those reported from other nearshore areas in northern Lake Michigan (Schelske and Callender 1970) and are indicative of extreme P limitation (Tilman 1982). Concentrations of PSi and chlorophyll were highest on 22 July and declined thereafter, indicating a decrease in phytoplankton standing crop (i.e. diatoms) typical of summer epilimnetic populations in Lake Michigan (Fahnenstiel and Scavia 1987a).

Estimates ($\bar{X} \pm$ SE) of conductivity (286.7 ± 2.1 $\mu mho \cdot cm^{-1}$ at 25°C), pH (7.97 ± 0.13), and dissolved oxygen (8.4 ± 1.3 $mg \cdot L^{-1}$) changed very little by depth and through time. Water transparency (Secchi depth = 5–6 m) also showed little temporal variability. Temperature profiles (20.2 ± 0.7°C) indicated that surface waters in the bay warmed very little during the study and that the thermocline was below 10 m (Carrick 1985).

Algal Biomass Estimates

Changes in algal biomass among nutrient treatments over all sampling periods as determined by chlorophyll, PSi, and total biovolume yielded consistent results (Table 2; Fig. 2). Samples on day 7 were not different in terms of total biovolume and PSi; however, chlorophyll concentrations were highest on Si + N + P substrata. By day 14, algal biomass was greatest on either Si + N + P (chlorophyll and PSi) or Si and Si + N + P treatments (biovolume). On day 31, all three biomass estimates were greater on N + P and Si + N + P substrata over CONT and Si treatments.

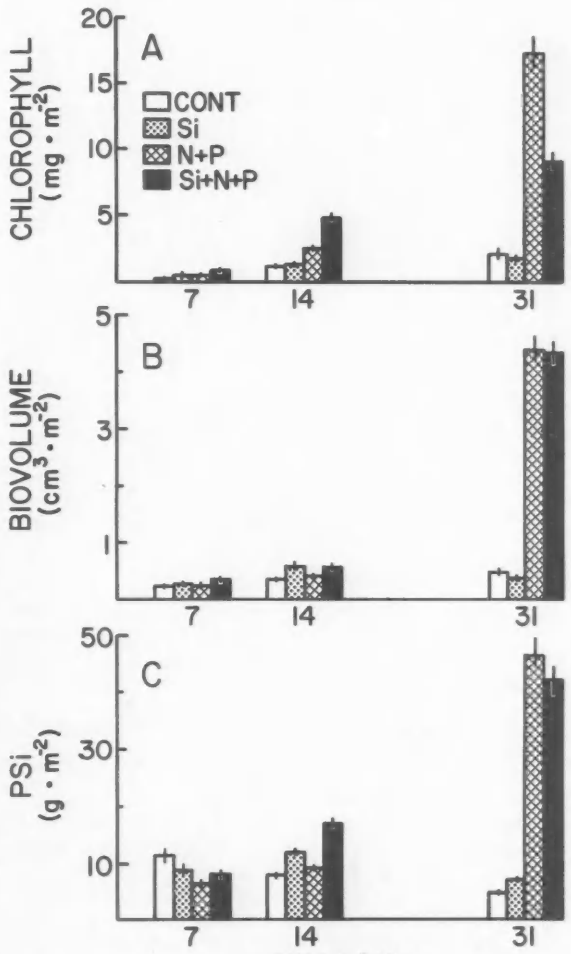

FIG. 2. Average (A) chlorophyll *a* concentrations, (B) total biovolume estimates, and (C) PSi concentrations among four nutrient treatments collected over three successive sampling periods. Vertical bars denote 1 SE from the mean.

Division-Level Responses

Biovolume of three divisions of algae (blue-green algae, green algae, and diatoms) did not vary significantly among nutrient treatments following 7 d of incubation (Table 3). After 14 d of exposure, diatom biovolume was highest on Si + N + P and Si substrata over the other two treatments. By day 31, blue-green and green algae both demonstrated a significant increase in biovolume on N + P and Si + N + P over Si and CONT substrata (Fig. 3).

TABLE 2. Results from one-way analysis of variance (ANOVA) comparing estimates of algal biomass among nutrient treatments for each sampling date. Treatments joined by underlining were not significantly different from one another when assessed with the Student–Newman–Keuls (SNK) multiple means range test (at $P < 0.05$). ***$P < 0.0001$.

Date	Biomass estimate	F (df = 3)	SNK range test
Day 7	Chlorophyll	13.29***	CONT N+P Si Si+N+P
	Biovolume	2.41	CONT N+P Si Si+N+P
	PSi	1.38	N+P Si+N+P Si CONT
Day 14	Chlorophyll	74.12***	CONT Si N+P Si+N+P
	Biovolume	13.35***	CONT N+P Si+N+P Si
	PSi	11.97***	CONT N+P Si Si+N+P
Day 31	Chlorophyll	29.22***	Si CONT Si+N+P N+P
	Biovolume	177.23***	Si CONT Si+N+P N+P
	PSi	140.18***	CONT Si N+P Si+N+P

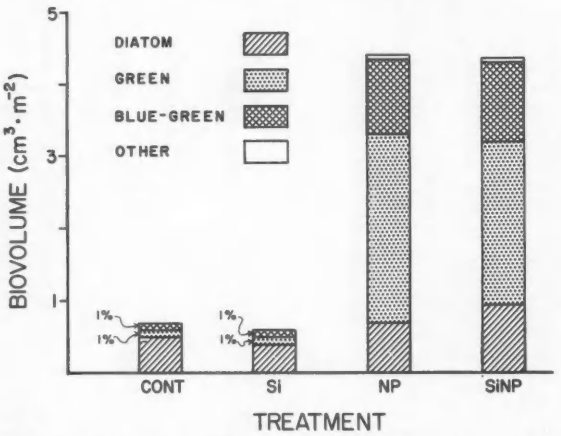

FIG. 3. Relative contribution of algal divisions to the total biovolume among four nutrient treatments after 31 d of exposure.

Population-Level Responses

The response of algal populations to nutrient enrichment following the 31-d incubation fell into four catagories: species responding positively to either Si, N+P, or Si+N+P and species responding negatively to all three patterns of enrichment (Table 4).

Eleven species of diatoms displayed significant growth response to Si enrichment (Table 4A) and accounted for between 1 and 2% of the total biovolume on Si substrata. *Amphora ovalis* (Kütz.) Kütz, *Cocconeis diminuta* Pant., *Cymbella minuta* Hilse ex Rabh., and *Nitzschia bacata* Hust. underwent from 1.2- to 3.7-fold increases in biovolume following Si enrichment. The biovolume of *Navicula cryptocephala* var. *veneta* (Kütz.) Rabh. and *Synedra rumpens* Kütz. was comparable on CONT and Si substrata but was less variable on Si substrata. The remaining five species only occurred on Si substrata.

Two species responded strongly to enrichment with N+P and Si+N+P (Table 4B; Fig. 4). The growth of *Schizothrix calcicola* (Ag.) Gomont, a small filamentous blue-green alga, was enhanced over 2000-fold on N+P and Si+N+P substrata and comprised a substantial portion of the total biovolume (24 and 26%, respectively). *Stigeoclonium tenue* (Ag.) Kütz., a green filamentous alga, increased more than 600-fold on N+P and Si+N+P substrata and accounted for 59 and 50% of the total biovolume in each community.

The diatoms *Achnanthes minutissima* Kütz. and *Cocconeis placentula* Ehr. responded moderately to enrichment with N+P, but increased to the greatest extent when all three nutrients were supplied (Table 4C; Fig. 4). *Achnanthes minutissima* underwent a 1.5-fold increase in biovolume on N+P substrata and a 2.3-fold increase on Si+N+P substrata (6 and 9% of the total biovolume, respectively). *Cocconeis placentula* showed a similar response to enrichment, increasing in biovolume 8.4-fold on N+P and more than 14-fold on Si+N+P substrata (4 and 6%, respectively).

Eleven species of diatoms maintained stable populations on CONT substrata and either decreased or were sporatically represented or absent on Si, N+P, and Si+N+P treatments (Table 4D). *Achnanthes clevei* var. *rostrata* Hust., *Cyclotella michiganiana* Skv., *C. ocellata* Pant., and *Nitzschia recta* Hantz. all decreased between 2.5- and 11.0-fold on Si substrata relative to controls and were absent on N+P and Si+N+P substrata. *Amphora hemicycla* Stoerm. & Yang only occurred on CONT substrata, while the remaining species had variable occurrences on N+P and Si+N+P compared with CONT and Si treatments. These species each contributed between 1 and 4% to the total biovolume on CONT and Si substrata.

Discussion

Enrichment with Si alone did not stimulate benthic algal growth to any significant extent in the nearshore area of northern Lake Michigan. However, Si may become a limiting factor to benthic algal growth following enrichment with N+P, as evidenced by the greatest increase in algal chlorophyll, biovolume, and PSi following Si+N+P enrichment during the first 14 d of the study. Enrichment with Si relative to N+P had little effect during the days 14–31, when factors other than nutrients seem to have become important.

It is quite possible that benthic algal communities at 10 m (ambient light is 4.5% of surface irradiance) are jointly limited by nutrients and light (see Rhee and Gotham 1981). This would explain the relatively low growth rates exhibited by the assemblage, despite the abundant supply of nutrients (see below). Moreover, chlorophyll to biomass ratios (micrograms chlorophyll per milligram, assuming specific weight = 1.0) were high on all treatments (average = 3.3) but greatest for N+P and Si+N+P treatments (3.6 and 3.9, respectively), indicating that these algae were the most light-limited. Ratios calculated here are comparable with those determined for shade-adapted subsurface phytoplankton existing under a similar light regime in Lake Michigan (Fahnenstiel and Scavia 1987b). In fact, subsurface phytoplankton demonstrated the greatest growth response to enrichment with Si and P only when higher than in situ light was provided (Fahnenstiel et al. 1984). Unexpectedly higher chlorophyll concentrations on N+P over Si+N+P substrata on day 31 may also be related to the greater contribution of green algae and subsequent chlorophyll *a* to that assemblage (see Fig. 3). Because green algae have a lesser pigmentation diversity than do blue-green algae and diatoms (Round 1981), they may contribute more chlorophyll *a* per unit cell volume.

The nearshore of Lake Michigan is a dynamic zone subject to periodic nutrient enrichment attributable to common upwell-

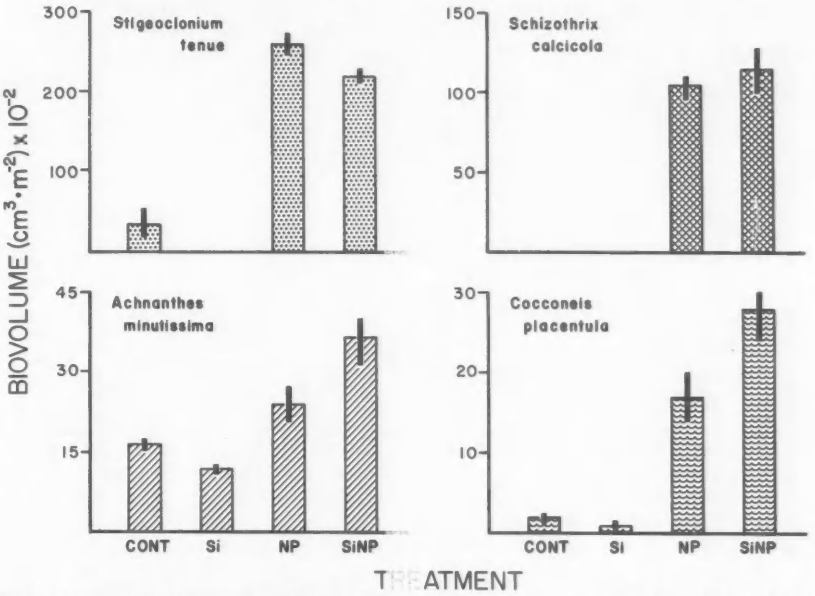

FIG. 4. Average biovolume of four dominant algal taxa among four nutrient treatment after 31 d of exposure. Vertical bars denote 1 SE from the mean.

TABLE 3. Results from one-way analysis of variance (ANOVA) comparing the biovolume of three algal divisions among nutrient treatments for each sampling date. Treatments joined by underlining were not significantly different from one another when assessed with the Student–Newman–Keuls (SNK) multiple means range test (at $P < 0.05$). ***$P < 0.0001$.

Date	Algal division	F (df = 3)	SNK range test
Day 7	Blue-green	3.17	CONT N+P Si+N+P Si
	Green	1.01	N+P Si CONT Si+N+P
	Diatom	2.32	CONT N+P Si Si+N+P
Day 14	Blue-green	1.81	N+P Si+N+P CONT Si
	Green	0.90	CONT N+P Si+N+P Si
	Diatom	13.95***	CONT N+P Si Si+N+P
Day 31	Blue-green	122.89***	CONT Si Si+N+P N+P
	Green	94.87***	Si CONT Si+N+P N+P
	Diatom	3.20	Si CONT N+P Si+N+P

ing events (Schelske et al. 1971), external perturbations (e.g. Auer et al. 1982), and inputs from tributaries (Schelske 1975). The release of both Si and P from substrata used here are comparable with Si and P concentrations in many Lake Michigan tributaries (Schelske 1975). Despite the relatively low atomic N:P provided by our substrata (3:1), it still is within the range of N:P measured from water entering Lake Michigan through the Grand River (Stephenson and Waybrant 1971). However, the supply of nutrients from nutrient-diffusing substrata can vary in time and space and potentially influence experimental results (Pringle 1987). Based upon nutrient-diffusion rates determined under laboratory conditions (Fairchild et al. 1985; this study), we assume that nutrients were supplied linearly throughout the experiment by our in situ substrata.

With this in mind, we feel that our results represent a realistic measure of the benthic algal response to nutrient perturbations relevant to the Lake Michigan system. Chlorophyll and particulate (biogenic) Si estimates for algal communities on N+P and Si+N+P substrata on day 31 fall well within the range for summer chlorophyll (11–77 $mg \cdot m^{-2}$) and biogenic concentrations (60 $g \cdot m^{-2}$, assuming 1 cm^3 = 1.1 g dry weight of sediments) present in nearshore southern Lake Michigan sediments (Nalepa and Quigley 1987; Conley et al. 1986, respectively). Also, our chlorophyll estimates correspond well with chlorophyll levels common in several oligotrophic Canadian lakes (Cattaneo 1987). Furthermore, according to the relationship between total P and periphyton biovolume for the same suite of Canadian lakes (Cattaneo 1987), the level of biovolume production we observed here correlates with water column total P concentrations (approximately 15–20 $\mu g \cdot L^{-1}$) common for nearshore Lake Michigan (Schelske 1980).

Aspects of Algal Community Development

The growth response of Lake Michigan benthic algae to nutrient enrichment is dependent upon the specific pattern of enrichment and on the period at which the community is analyzed. This temporal interaction may reflect the operation of three components of algal community development: colonization, growth, and maturation. Collections on day 7 can be interpreted to represent a period of algal colonization and acclimation (average exponential growth rate, $r = 0.33 \cdot d^{-1}$), where algal biomass differed little among treatments. Values for r during this period may be inflated because passive accumulation was not taken into account (Bothwell and Jasper 1983). Days 7–14 can be characterized as an initial growth phase ($r = 0.19 \cdot d^{-1}$), where increases in biomass were highest on Si+N+P substrata and community composition was similar among treatments. Days 14–31 represent a period where algal biomass accumulation on N+P and Si+N+P treatments increased nearly ninefold over Si and control treatments and

TABLE 4. Species showing significant biovolume ($cm^3 \cdot m^{-2} \times 10^{-3}$) responses to specific nutrient additions on day 31 ($\bar{X} \pm$ SE), as determined by one-way analysis of variance. Species responses were classified into groups using the Student–Newman–Kuels (SNK) range test ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Species	CONT	Si	N+P	Si+N+P
		A. Species responding to Si enrichment		
*Achnanthes exigua**	0	0.5 ± 0.5	0	0
*Amphora ovalis****	2.0 ± 2.0	7.5 ± 0.9	0	0
*Cocconeis diminuta**	1.0 ± 0.6	1.2 ± 0.4	0	0
*Cymbella hustedtii**	0	1.6 ± 0.7	0	0
*C. minuta****	1.0 ± 0.6	2.7 ± 0.7	0	0
*Diploneis oculata***	0	1.1 ± 0.4	0	0
*Gomphonema gracile**	0	1.3 ± 0.8	0	0
Navicula cryptocephala var. *veneta****	1.8 ± 0.3	1.8 ± 0.2	2.1 ± 2.1	0
*Nitzschia acicularis****	0	1.3 ± 0.6	0	0
*N. bacta**	1.3 ± 0.8	1.7 ± 0.6	0	0
*Synedra rumpens**	0.7 ± 0.4	0.7 ± 0.2	0	0
		B. Species responding to N+P enrichment		
*Schizothrix calcicola****	0.4 ± 0.4	0	1043.6 ± 129.1	1130.9 ± 367
*Stigeoclonium tenue****	3.5 ± 3.5	0	2578.5 ± 251.6	2201.7 ± 164
		C. Species responding to Si+N+P enrichment		
*Achnanthes minutissima**	161.8 ± 26.5	120.1 ± 16.9	243.6 ± 60.9	283.1 ± 63
*Cocconeis placentula****	20.1 ± 3.6	11.9 ± 3.3	169.4 ± 59.1	376.1 ± 108
		D. Species responding negatively to enrichment		
Achnanthes clevei var. *rostrata**	1.4 ± 0.7	0.1 ± 0.1	0	0
*Amphora hemicycla***	8.0 ± 3.8	0	0	0
*Cyclotella comensis****	4.9 ± 1.5	1.1 ± 0.3	0.5 ± 0.5	0
*C. michiganiana****	1.2 ± 0.4	0.5 ± 0.5	0	0
*C. ocellata****	1.5 ± 0.7	0.4 ± 0.1	0	0
*Fragilaria construens***	10.4 ± 5.9	5.5 ± 2.9	0	0
F. construens var. *pumila**	3.9 ± 2.1	0.4 ± 0.4	0	0
*F. crotonensis****	19.0 ± 3.3	10.4 ± 4.3	53.8 ± 53.8	0
*F. intermedia**	4.8 ± 2.8	2.1 ± 1.3	0	0
*Nitzschia palea****	7.2 ± 2.5	2.5 ± 1.2	0	1.9 ± 1.9
*N. recta**	8.8 ± 3.1	2.6 ± 1.5	0	0

consisted mainly of four species. Exponential growth rates were highest for N+P and Si+N+P treatments ($r = 0.11$ and $0.04 \cdot d^{-1}$), but decreased on all treatments compared with days 7–14 (average $r = 0.05 \cdot d^{-1}$). This decline in growth rate for all treatments suggests that each assemblage reached some abundance plateau (Rodriguez 1987). Overall, the algal growth rates following enrichment in this study are very low compared with those obtained for Lakes Huron and Michigan phytoplankton assemblages enriched with Si, N, and P (average r for 23 July 1975 = $0.44 \cdot d^{-1}$; Lin and Schelske 1981) and with Si and P (average $r = 0.40 \cdot d^{-1}$; Schelske et al. 1975), respectively. Furthermore, lotic periphyton exposed to various concentrations of effluent high in N and P experienced increases in intrinsic growth rates (average r for all concentrations = $0.35 \cdot d^{-1}$) over controls (average $r = 0.16 \cdot d^{-1}$) (Bothwell and Stockner 1980).

Alterations in Community Composition

Following an increase in diatom biovolume for the first 14 d of the experiment, the community composition on substrata enriched with N+P and Si+N+P shifted to one dominated by green and blue-green algae. This shift in algal community structure is similar to changes observed from in situ bag experiments involving Lake Michigan phytoplankton assemblages exposed to a similar pattern of nutrient enrichment (Schelske and Stoermer 1971, 1972). Such alterations in phytoplankton community structure were attributed to depletion of soluble Si within the phytoplankton enclosures.

Mechanisms prompting taxonomic shifts in the present study are difficult to ascribe. Low N:P like those supplied by our substrata have been shown to favor the growth of green and blue-green benthic algae in another lake enrichment study (Schindler 1975). Also, the ability of filamentous green and

blue-green algae to take up large stores of nutrients (Rosemarin 1982; Stewart et al. 1978, respectively), suffer minimal grazing losses (Canale and Auer 1982), and reduce shading effects (Cattaneo 1987) cannot be discounted as factors contributing to their relative success on N + P and Si + N + P treatments.

Enrichment of plant communities in restricted growing space often escalates the importance of resources other than nutrients (i.e. light and space) and the interactions among species utilizing these resources (Tilman 1982). Given the low light conditions and high algal standing crop on N + P and Si + N + P substrata by day 31, comparable development of biomass and community composition on N + P and Si + N + P substrata at the end of the experiment may have been mediated, in part, by the relative success of a few dominant species.

Communities on N + P and Si + N + P substrata consisted primarily of four species. *Schizothrix calcicola* (Cyanophyta) and *Stigeoclonium tenue* (Chlorophyta) formed extensive mats covering most of the surface of N + P and Si + N + P substrata. In addition, Fairchild et al. (1985) also identified *S. tenue* as a dominant following enrichment with N + P in Douglas Lake, Michigan, and commented on the possibility of habitat modification by this species. The other two dominant taxa on these treatments (*Achnanthes minutissima* and *Cocconeis placentula*) are adnate diatoms of very limited motility (Patrick and Reimer 1966) which thrive in habitats of limited space (Pringle 1985; Krejci and Lowe 1986) and light (Stockner and Shortreed 1978; McIntire 1968, respectively). Hence, we feel that secondary limitation of light and space were pertinent forces under these two nutrient regimes by day 31. Many algal species on these treatments appeared to vary as a function of the relative success of *Schizothrix* and *Stigeoclonium*, and only species with low requirements for light and space coexisted (primarily *Achnanthes* and *Cocconeis*).

Population Responses to Nutrient Enrichment

The observed growth enhancement of 11 diatom species following Si enrichment suggests that these species were Si limited. Of these, *Cocconeis diminuta*, *Nitzschia bacata*, and *Nitzschia acicularis* are known to thrive in nutrient-enriched habitats (Lowe 1974). Also, *Nitzschia acicularis* grows best in cultures with high Si concentrations (approximately 30 $mg \cdot L^{-1}$; Chu 1942). No specific data pertaining to the nutrient requirements of the other eight diatom species are available. Because some Si is liberated from the CONT substrata, estimates of algal response to Si enrichment are probably conservative.

In addition to ambient dissolved Si concentrations, benthic diatoms may be able to use other sources of Si. Diatoms may be able to sequester Si by modifying the biogeochemical decomposition of silicate minerals (Hutchinson 1957). Studies by Vernadsky (1922) have documented the breakdown of silicate materials (i.e. kaolinite and nacrite) by species of the diatom genera *Nitzschia* and *Navicula*. Also, significant release of Si from nearshore sediments in Lake Michigan (Quigley and Robbins 1984) may be another potential Si source. Third, the high annual sedimentation of remnants from the spring diatom bloom in Lake Michigan (Fahnenstiel and Scavia 1987a) settles in some nearshore zones (Nalepa and Quigley 1987) and deposits diatom frustules and fragments which can dissolve swiftly (80–100% annually; Conway et al. 1977), providing another potential Si source. Thus, benthic diatoms may have a mode of maintaining populations in Si-poor waters by using an additional source(s) of Si present in the sediments (Cheng and Tyler 1973).

The increase in algal biomass on N + P and Si + N + P substrata was primarily due to the growth of *Stigeoclonium tenue* and *Schizothrix calcicola*. *Stigeoclonium tenue* grows well in organically enriched habitats (Palmer 1969). In addition, culture studies have demonstrated increased growth response of *S. tenue* to enrichment with combinations of NO_3^- and PO_4^{3-} (DeVries et al. 1985) and a capacity for high P-uptake kinetics and growth rates ($2.0 \cdot d^{-1}$) (Rosemarin 1982). *Schizothrix calcicola* is also associated with organic pollution (Van Landingham 1983), and is one of the more pollution tolerant members of the Oscillatoriaceae (Drouet 1968). Because green and blue-green algae have little to no requirement for Si (Hutchinson 1957), we deduce that *Schizothrix* and *Stigeoclonium* responded to supplies of N and P (and/or other unknown ions) liberated from N + P and Si + N + P substrata.

The diatoms *Achnanthes minutissima* and *Cocconeis placentula* experienced increased growth on N + P substrata and reached the greatest abundance on substrata enriched with Si + N + P. Although *A. minutissima* is found in a variety of freshwater habitats (Lowe 1974), it responds to nitrate addition (Stockner and Shortreed 1978; Fairchild and Lowe 1984; Pringle and Bowers 1984), as does *Cocconeis* (Pringle and Bowers 1984; Fairchild et al. 1985). Our results indicate that both species respond positively to N + P addition and that greatest growth is obtained when Si is also provided.

The negative response to nutrient enrichment exhibited by 11 diatom species was expected given what is known about their autecology. The three *Cyclotella* species encountered here have been characterized as pollution-intolerant species (Stoermer 1978) which decline in abundance under increasing eutrophic conditions (Stoermer et al. 1985). Of the four sensitive species of *Fragilaria*, *F. construens* and *F. construens* var. *pumila* are intolerant to eutrophication (Engstrom et al. 1985). *Fragilaria crotonensis* is a more tolerant "weed" species that occurs under a wide range of conditions and declines under more advanced stages of nutrient perturbation (Stoermer et al. 1985). *Nitzschia palea* is also regarded as a "weed" species (Lowe 1974) and perhaps responds to nutrient enrichment much like *F. crotonensis*. The cosmopolitan nature of *F. crotonensis* and *N. palea* may account for their variable response to nutrient enrichment in this study. Information concerning the autecology of the other sensitive species is scarce.

Concluding Remarks

In summary, enrichment with Si + N + P led to the greatest increase in total algal chlorophyll, biovolume, and PSi between treatments during the first 14 d of the study when diatoms dominated the assemblage. Additions of Si alone affected a limited number of poorly represented species. Thus, Si appears to be a secondarily limiting nutrient, becoming important to benthic algae following N + P addition. Although the observed shift from diatom to green and blue-green dominance following N + P enrichment in this study coincides with findings for Lake Michigan phytoplankton exposed to the same pattern of enrichment (Schelske and Stoermer 1971, 1972), the mechanism driving these changes may differ. Taxonomic shifts observed here were attributed to the low N:P supplied and to species-level interactions. Low growth rates and high chlorophyll to biomass ratios for algae across treatments is indicative of light limitation, which was apparently accentuated by the extensive growth of *Schizothrix* and *Stigeoclonium* on N + P and Si + N + P substrata. This implies that the trade-off between nutrients and other potentially limiting resources (i.e. light and space) cannot be

ignored and may manifest themselves readily within communities restricted to substrata.

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Sequential Decision Plans in Monitoring Benthic Macroinvertebrates: Cost Savings, Classification Accuracy, and Development of Plans

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Jackson, J. K., and V. H. Resh. 1988. Sequential decision plans in monitoring benthic macroinvertebrates: cost savings, classification accuracy, and development of plans. Can. J. Fish. Aquat. Sci. 45: 280–286.

Sequential decision plans provide a statistical approach that can reduce the number of benthic sample units needed to classify a site as impacted or unimpacted, thus reducing the cost of using benthic macroinvertebrates in water quality assessment programs. These plans require information about unimpacted and impacted conditions, the mathematical distribution of the data, and acceptable risks of classification error. A large benthic data set (n = 55) was used for simulations that created and tested sequential decision plans. Using 10–60% reductions in species richness, mayfly (*Cinygmula*) population density, and species diversity as definitions of impact in the simulations, the average number of sample units processed for identification of the unimpacted reference site was reduced (compared with fixed sample-size methods that are commonly used) by 50–64% for species richness, 59–79% for density estimates, and 51–55% for species diversity. Unimpacted data sets were initially classified as representing impacted conditions in 0–5% of the cases. If classifications are to be interpreted properly, sampling error and spatial and temporal variation in biological parameters must be considered when sequential decision plans are created.

Les plans séquentiels de décision fournissent une approche statistique qui peut réduire le nombre d'unités d'échantillonnage du benthos nécessaires à la classification d'endroits comme perturbés ou non perturbés. On verrait ainsi une baisse des coûts qu'entraîne l'utilisation d'échantillons de macroinvertébrés benthiques dans le cadre de programmes d'évaluation de la qualité de l'eau. Ces plans requièrent des données sur les conditions liées à la perturbation et à la non-perturbation, la répartition mathématique des données et les risques acceptables liés à une erreur de classification. On a utilisé une importante série de données sur le milieu benthique (n = 55) dans des simulations qui ont vu la création et la vérification de plans séquentiels de décision. En utilisant des baisses variant de 10 à 60 % de la richesse spécifique, la densité de la population d'éphémères (*Cinygmula*) et la diversité des espèces comme définitions de l'impact, on a obtenu une baisse du nombre moyen d'unités d'échantillonnage nécessaires à l'identification du site étalon non perturbé (par rapport aux méthodes communément utilisées requérant un nombre fixe d'échantillons); cette baisse a varié de 50 à 64 % dans le cas de la richesse spécifique, de 59 à 79 % pour ce qui est des estimations de la densité et de 51 à 55 % dans le cas de la diversité des espèces. Les séries de données sur les endroits non perturbés ont initialement été classées comme représentant un endroit perturbé dans 0 à 5 % des cas. Si l'on veut obtenir une interprétation adéquate des classifications, on doit tenir compte de l'erreur d'échantillonnage et des variations spatiales et temporelles des paramètres biologiques au moment de l'élaboration de plans séquentiels de décision.

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The environmental impact assessment process has two components, the predevelopment predictive phase and the postdevelopment monitoring and assessment phase. The first phase uses field surveys, experimentation, and models to form predictions about the effect of a proposed project on environmental quality. The second phase provides the basis for recognizing environmental changes that are associated with the project; in addition, this phase evaluates the validity of development-related impact predictions (Rosenberg et al. 1981, 1986).

The detection of changes in environmental quality depends on the accuracy and precision of the data obtained in the monitoring program. Accuracy describes how well the data represent true field conditions, whereas precision refers to the consistency and reliability of the sample estimators and is usually expressed as confidence intervals (Fowler and Witter 1982). Although accuracy is essential to biological monitoring, precision has received the most attention because it underlies our statistical ability to separate natural variation from development-induced changes in environmental quality.

Of all freshwater plants and animals, aquatic macroinvertebrates have been recommended most often for use in the biological monitoring of water quality (Hellawell 1977, 1978). Because of natural variation in the distribution of benthic organisms, large sample sizes are often required to achieve reasonable levels of precision in benthic studies (Needham and Usinger 1956; Downing 1979; Resh 1979; Morin 1985). However, it is the time spent processing each sample unit (i.e. the sepa-

ration of organisms from substrate, followed by identification and enumeration of organisms), rather than the time spent collecting these sample units, that often poses a practical limit on sample sizes. (Note: In this paper, a sample consists of n sample units (sensu Snedecor and Cochran 1967; Elliott 1977).) Time and cost limitations often result in sampling programs that have insufficient numbers of reference (control) and impacted (treatment) sites (Eberhardt 1976), insufficient numbers of sample units per site (Resh 1979), and pre- and postdevelopment monitoring periods that are of inadequate duration (Rosenberg et al. 1981). These deficiencies contribute to the inaccuracy and imprecision of benthic studies.

The concept of sequential decision plans (which is also referred to as sequential sampling or sequential analysis, but which is different than the sequential counting plans of Kuno (1969) and Allen et al. (1972)) was developed by Wald (1945, 1947) as a means of reducing the average number of sample units needed to describe a study subject (e.g. a study site) compared with the number required using fixed sample-size procedures. With fixed sample sizes, all collected sample units are examined and statistical parameters (e.g. means, variances) are estimated; these estimates are then used to classify the data sets. In contrast, when using a sequential decision plan, the data are classified into predetermined categories (e.g. unimpacted, impacted, or no-decision) after each sample unit is examined, and statistical parameters are not estimated. Fewer sample units are examined with sequential decision plans because sample analysis stops when the minimum information needed for making an unimpacted/impacted classification is obtained. Sequential decision plans have been used successfully in monitoring forest and agricultural pests (Poole 1974; Southwood 1978; see Pieters 1978 for bibliography), in aquatic microbiology (Maul et al. 1985), and in other environmental studies (e.g. Green 1979). Their use in assessing environmental impact with freshwater macroinvertebrates was recently illustrated in two scenarios by Resh and Price (1984).

In this study, we have created a series of sequential decision plans that use benthic macroinvertebrate species richness, population density, and species diversity to test the applicability of this approach to water quality monitoring. Our goals were to (1) demonstrate the potential cost savings (as time) of using sequential decision plans rather than fixed sample-size programs in benthic monitoring programs, (2) determine if sequential decision plans created from small data sets (which are characteristic of benthic monitoring programs that have sample sizes set by fiscal limitations) can correctly identify environmental conditions (e.g. unimpacted compared with impacted conditions), and (3) identify economically feasible classification categories.

Sequential Decision Plans

A priori Choices for Sequential Decision Plans

Three essential elements are defined before a sequential decision plan is created: (1) classification thresholds are set (classification thresholds describe the boundaries of the classification categories (e.g. impacted, unimpacted, and no-decision)), (2) the mathematical distribution of the data (e.g. normal, Poisson, negative binomial) is determined, and (3) acceptable probabilities of classification errors (Type I and II errors) are assigned.

Classification threshold definitions (also called class limits) are developed using available ecological information to relate

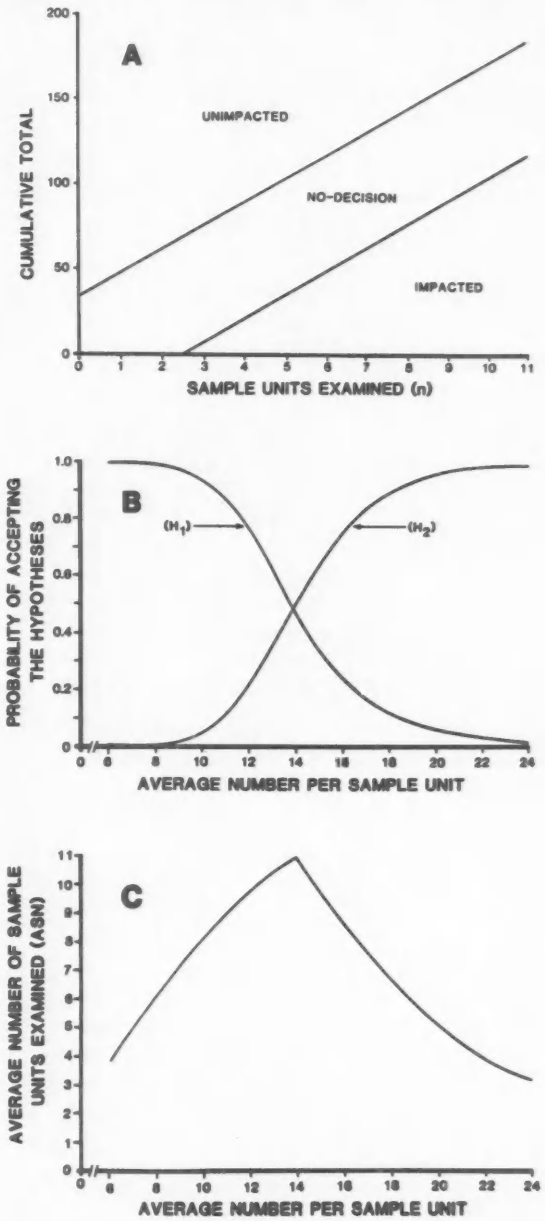

FIG. 1. (A) Sequential decision plan for monitoring the density of a hypothetical macroinvertebrate population whose density decreases under impacted conditions. The upper classification threshold (designating no impact) = 20 individuals per sample unit; the lower classification threshold (designating impact) = 10 individuals per sample unit. These thresholds are expressed as parts of the following hypotheses: H_1: impacted conditions exist when mean density is ≤ 10 individuals per sample unit; H_2: unimpacted conditions exist when mean density is ≥ 20 individuals per sample unit. Parameter $k = 2$ and $\alpha = \beta = 0.05$. (B) Operating characteristic (OC) curve and (C) average sample size (ASN) curve of the sequential decision plan illustrated in Fig. 1A.

the measured variable (e.g. species richness) to impacted and unimpacted conditions. The thresholds are often expressed as parts of hypotheses that describe the classification categories (see Fig. 1). Classification threshold definitions may be affected by cost considerations that limit the maximum number of sample units processed.

The mathematical distribution of a data set is usually described in terms of known model distributions. Sequential decision plans can be developed using data that conform to the negative binomial, binomial, Poisson, or normal distributions (Waters 1955; Onsager 1976).

Two types of classification errors can occur when sequential sampling plans are used. A Type I error (probability = α) occurs when a data set is classified into the impacted category when it actually belongs in the unimpacted category. A Type II error (probability = β) occurs when a data set is classified into the unimpacted category when it actually belongs in the impacted category. Type I errors could have economic implications if mitigation activities were started even though unnecessary. Type II errors could have environmental implications because the misclassification would not be detected until the next sampling period and continued degradation could result in more severe impact.

Creation of a Sequential Decision Plan

The decision lines in a sequential decision plan describe the three classification categories (unimpacted, no-decision, and impacted) in terms of cumulative totals at various sample sizes (Fig. 1A). The *y*-intercept and the slope of each decision line are calculated using the classification thresholds, α, β, and (when the normal or negative binomial distributions apply) the sample variance or the dispersion index k (see Appendix for formulae). There are two additional features of the sequential decision plan that can be calculated: the operating characteristic (OC) curve and the average sample size curve (the acronym of which was euphemistically changed to ASN in the 1940's). The formulae for these two features are based on work by Wald (1945, 1947); Fowler (1983) showed that Wald's original formulae are appropriate for small data sets, which are common in benthic studies.

The OC curve illustrates the relationship between the sample mean (average number per sample unit) and the probability of accepting either hypothesis, only one of which is correct (Fig. 1B). It is calculated using α, β, the classification thresholds or the *y*-intercepts of the decision lines, and, when appropriate, the sample variance or k (see Appendix for formulae). The variation in error risk shown in the OC curve is used in the calculation of the ASN curve.

The ASN curve illustrates how the average number of sample units examined varies with the sample mean (average number per sample unit; Fig. 1C). It is calculated using the decision lines from the sequential decision plan and the error risks from the OC curve (see Appendix for formulae). The maximum point of the ASN curve (the maximum ASN) is often used to define the number of sample units collected in the field (i.e. the maximum sample size examined). As the sample mean becomes larger or smaller than the mean associated with the maximum ASN, both the risk of misclassification and the average number of sample units that are actually examined for classification decrease (Fig. 1B, 1C).

Detailed examples of sequential decision plan classifications can be found in Pieters and Sterling (1974) and Resh and Price (1984).

Methods

Simulations Using Field Data

Aquatic macroinvertebrate data from Needham and Usinger (1956) were used in this study. This is a large (originally 100 benthic sample units), well-known data set that has been used in various analyses (e.g. Chutter 1972; Resh 1979). The sampling procedures and the levels of taxonomic identification reported by Needham and Usinger (1956) are still typical of present-day benthic monitoring studies. Simulations were done by using the Needham and Usinger data set to create and test sequential decision plans.

In creating a plan, 15 sample units were drawn randomly from the Needham and Usinger data set (which was reduced to $n = 55$ after Chutter's (1972) suggestions) and the sample mean and variance were calculated. The upper classification threshold (designating unimpacted conditions) was defined as $\bar{X} - 2SE$; the lower limit of the confidence interval (i.e. $-2SE$) was used as a conservative adjustment of the effects of sampling error on the sample mean. The lower classification threshold (designating impacted conditions) was defined as a 10, 20, 30, 40, 50, or 60% reduction of the no-impact threshold; this approach to designating impact is currently used or being considered by several U.S. states (e.g. Florida Administrative Code, Chapter 17-3, Water Quality Standards). The mathematical distribution was calculated for each plan. Error risks α and β were defined as 0.05.

The sequential decision plan that was created from each subset of 15 sample units was tested by randomly selecting from the 40 sample units that remained in the Needham and Usinger data set. Data from each observation were cumulatively totaled, and testing ceased when an unimpacted/impacted classification occurred or when a predetermined maximum sample size was reached. Maximum sample size was defined either as the maximum point on the ASN curve, or as 15 sample units, whichever was less. We arbitrarily chose 15 sample units as the maximum number of benthic sample units that would be practical to examine in classifying sites for benthic impact assessment programs. If the sample cumulative total remained in the no-decision category after the specified maximum number of sample units were examined, the sample was classified as a no-decision. The truncated decision method, which created a single decision line by dividing the no-decision zone in half and then adding the halves to the adjacent classification categories, was used to reclassify these no-decision cases (Wald 1947).

For each of three biological variables, the creation and testing of sequential decision plans was repeated 20 times for each of the six impact thresholds (360 simulations total). In the first series of analyses, species richness (the number of species per 0.093 m^2) was chosen as the biological variable because it is the most rapidly estimated community parameter and it is often sensitive to changes in water quality (e.g. Dills and Rogers 1974; Hellawell 1977). The second biological variable chosen, population density (using the mayfly *Cinygmula* expressed as number of individuals per 0.093 m^2), illustrates the use of a single population for biomonitoring (e.g. the approach used by Resh and Unzicker (1975), Rosenberg et al. (1977), and Resh et al. (1981)). This mayfly was chosen because the population probably represented a single species, it was relatively abundant in Prosser Creek, and mayflies often have been shown to be sensitive to changes in water quality (Hubbard and Peters 1978). Finally, species diversity (per 0.093 m^2) was examined because

this variable is used widely in benthic monitoring. The index chosen, Inverse Simpson's Dominance, is reported to be a reliable indicator of environmental impact (Hellawell 1977) and, unlike most diversity or biotic indices, its mathematical distribution is known (Patil and Taillie 1976; Routledge 1979). This latter feature allows for data analysis with parametric statistics.

For each plan, a fixed sample size (n) was calculated for the mean used to define the upper classification threshold and for the mean represented by the lower classification threshold. Sample size formulae appropriate for each mathematical distribution were from Elliott (1977). The difference between these two means was used in an iterative process to create nonoverlapping 95% confidence intervals that produced equal fixed sample sizes. Time savings was then calculated as (mean fixed sample size − mean sample units examined) ÷ mean fixed sample size × 100%.

The accuracy of the sequential decision plan classifications could be assessed because each subset tested was part of the same, unimpacted Needham and Usinger data set; therefore, a classification that indicated impacted conditions was a classification error.

Results

Monitoring Species Richness

Species richness ranged from 7 to 20 species per 0.093 m² in Needham and Usinger's (1956) original data. Mean species richness for our 20 simulations of unimpacted data ranged from 12.1 to 13.8 species per sample unit. Because species richness can be assumed to have a Poisson distribution (Patil and Taillie 1976), the Poisson formulae for sequential decision plans were used in our calculations. The no-impact thresholds ranged from 10.6 to 12.7 for the different simulations. In this and the analyses below, the impact thresholds represented a 10–60% reduction of the no-impact thresholds.

The results of the simulations are grouped according to their impact threshold definitions (Table 1A). The sample size estimators (i.e. maximum ASN, fixed sample size, and the mean number of sample units that had to be examined for a classification) decreased as the difference between the impact and no-impact thresholds increased from 10 to 60%. Mean time savings that resulted from using sequential decision plans were 50–64% for all definitions of the impact threshold. Initial classifications were correct in at least 80% of the cases at all impact threshold definitions except the 10% reduction threshold. None of the initial classifications were incorrect. Initial classifications were no-decisions in 20% or less of the cases at all impact thresholds except the 10% reduction threshold. After using the truncated decision method to reclassify the no-decision classifications, 95–100% of all classifications were correct (Table 1A).

Monitoring Population Density

Population density of *Cinygmula* ranged from 0 to 37 individuals per 0.093 m² in Needham and Usinger's (1956) original data. In the 20 simulations from these data, mean density of *Cinygmula* ranged from 9.5 to 17.3 individuals per 0.093 m². Estimates of the parameter k were generally between 2 and 4 (range = 1.60–8.52; median = 2.90); because sample estimates of population density for the mayfly *Cinygmula* conformed to the negative binomial series, the formulae for negative binomial data were used to create the sequential decision plans. The no-impact thresholds ranged from 5.7 to 12.6. As before, impact thresholds represented a 10–60% reduction of the no-impact thresholds.

Similar to the results for species richness, the sample size estimators (i.e. maximum ASN, fixed sample size, and the mean number of sample units that had to be examined for a classification) decreased as the difference between the impact and no-impact thresholds increased from 10 to 60% (Table 1B). At all definitions of the impact threshold, mean time savings that resulted from using sequential decision plans were between 59 and 79%. Initial classifications were correct in at least 75% of the cases at all impact threshold definitions except the 10% reduction threshold. Before using the truncated decision method, classification errors were 5% or less. The truncated decision method correctly reclassified 71% of the no-decision classifications; thus, 80–95% of all classifications were correct (Table 1B).

Monitoring Species Diversity

Species diversity for the individual sample units in Needham and Usinger's (1956) original data ranged from 2.9 to 10.4. Mean species diversity for our 20 simulations from these data ranged from 5.7 to 7.4. Sample variance was generally small in our data sets (variance range = 1.9–5.1; coefficient of variation = 19.7–38.2%). Formulae for normally distributed data were used to create sequential decision plans because the Inverse Simpson's Dominance index conforms to the normal distribution (Patil and Taillie 1976; Routledge 1979). The no-impact thresholds ranged from 4.8 to 6.4.

As the difference between the impact and no-impact thresholds increased from 10 to 60%, sample size estimators (i.e. maximum ASN, fixed sample size, and the mean number of sample units that had to be examined for a classification) decreased (Table 1C). Mean time savings that resulted from using sequential decision plans were greater than 50% for all definitions of the impact threshold. Before using the truncated decision method, at least 75% of the cases were classified correctly except when the impact threshold was a 10% reduction. Likewise, classification errors never exceeded 5% for any impact threshold definition before using the truncated decision method. The truncated decision method correctly reclassified 73% of the no-decision classifications; thus, 90–95% of the classifications were correct (Table 1C).

Discussion

Reduced Sampling Effort

Resh and Price (1984) proposed that sequential decision plans could provide correct descriptions of environmental conditions based on fewer sample units than fixed sample-size procedures. As a result, monitoring costs would be reduced. Time savings in our simulations were from 50 to 79%. Savings could be significantly larger or smaller, depending on the decision plan and field conditions. For example, if biotic changes resulting from impact are pronounced, relatively few sample units would be examined before sites are classified as impacted; in this case, time savings would be great. Alternatively, if impact effects are slight, the mean number of sample units examined before classification occurs would be greater and could approach the sample size used in fixed sample-size programs. In field studies of crop pests, 20–80% fewer sample units were examined using

TABLE 1. Sample size estimators (mean maximum ASN, mean conventional sample size, mean sample units examined per classification), mean time saved, and percentage of 20 classifications that were correct, incorrect, no-decisions, and correct after using the truncated decision method.

	Definition of impact threshold					
	10% reduction	20% reduction	30% reduction	40% reduction	50% reduction	60% reduction
(A) Results from sequential decision plans using species richness						
Maximum ASN	71.1	16.8	7.0	3.7	2.2	1.4
Fixed sample size	34.5	15.1	8.3	5.1	3.4	2.3
Sample units examined (≤15)	12.3	7.6	3.4	2.0	1.5	1.0
Time saved (%)	64.3[a]	49.7	59.0	60.8	55.9	56.5
Correct classifications (%)	45.0	100.0	95.0	85.0	80.0	90.0
Incorrect classifications (%)	0.0	0.0	0.0	0.0	0.0	0.0
No-decision classifications (%)	55.0	0.0	5.0	15.0	20.0	10.0
Correct classifications with truncation (%)	100.0	100.0	100.0	95.0	100.0	100.0
(B) Results from sequential decision plan simulations using population density of Cinygmula						
Maximum ASN	357.0	81.2	32.7	16.4	9.2	5.6
Fixed sample size	33.5	23.1	16.8	12.7	9.9	7.8
Sample units examined (≤15)	13.7	9.2	6.7	3.4	2.1	1.8
Time saved (%)	59.1[a]	60.2[a]	60.1[a]	73.2	78.8	76.9
Correct classifications (%)	35.0	75.0	85.0	90.0	90.0	90.0
Incorrect classifications (%)	0.0	0.0	0.0	5.0	5.0	5.0
No-decision classifications (%)	65.0	25.0	15.0	5.0	5.0	5.0
Correct classifications with truncation (%)	80.0	95.0	95.0	95.0	95.0	90.0
(C) Results from sequential decision plan simulations using species diversity						
Maximum ASN	103.1	25.8	11.5	6.3	4.1	2.9
Fixed sample size	27.8	15.1	9.4	6.5	4.7	3.6
Sample units examined (≤15)	13.0	7.4	4.3	2.9	2.3	1.7
Time saved (%)	53.2[a]	51.0[a]	54.3	55.4	51.1	52.8
Correct classifications (%)	50.0	85.0	85.0	85.0	85.0	75.0
Incorrect classifications (%)	0.0	5.0	5.0	5.0	5.0	5.0
No-decision classifications (%)	50.0	10.0	10.0	10.0	10.0	20.0
Correct classifications with truncation (%)	90.0	95.0	90.0	90.0	90.0	90.0

[a]Time-savings estimate was influenced by limiting the maximum number of sample units examined to 15.

sequential decision plans compared with fixed sample-size programs (e.g. Pieters and Sterling 1974; Nilakhe et al. 1982).

In our sequential decision plan simulations, the smallest impact that could be economically identified was a 20% reduction in species richness, a 30% reduction in species diversity, or a 40% reduction in population density of *Cinygmula*. Attempting to identify smaller impacts resulted in sequential decision plans with maximum ASN's that were far larger than our practical maximum sample size definition (15 sample units). A few of the economically feasible plans required that an average of two to three additional sample units be collected over the number required in fixed sample-size designs (Table 1). This does not affect the cost of using sequential decision plans greatly because (1) the time involved in collecting a benthic sample unit is a fraction of the time involved in examining that sample unit (Resh and Price 1984), (2) these additional sample units would be examined rarely (Table 1), and (3) many water quality consultants already collect additional sample units that are saved for future needs.

Maximum Sample Size Reduction

We were able to reduce the maximum number of sample units

to be collected in the field (the maximum ASN) to a practical sample size (i.e. 15 sample units or less) by changing the impact threshold definition (e.g. from a 20 to a 30% reduction). If classification threshold definitions cannot be changed (e.g. due to legal definitions of impact such as a 25% reduction in species diversity in Florida Administrative Code, Chapter 17-3, Water Quality Standards), two other approaches can be used to reduce maximum sample size. First, the risk of classification error can be increased. Although both α and β can be increased, if either error is considered less important than another, then only the less important error risk would be increased. Second, a sequential decision plan that has a large maximum ASN can be used but the maximum sample size collected is reduced to a cost-effective number (e.g. mean maximum ASN was 26 in the 20% reduction of species diversity example (Table 1C) but only 15 sample units were collected). This alternative can result in an increase in no-decision classifications and more frequent use of the truncated decision method. Classifications using the truncated decisions method were 71–95% correct in our study and 75% correct in the study by Connola et al. (1959).

Final Considerations

Two topics that are important in the use of sequential decision plans in benthic monitoring should be addressed: the effects of (1) sampling error and (2) spatial and temporal variability on the applicability of classification thresholds.

True sampling error is the difference, due to chance alone, between the sample estimators (i.e. means, variances, or the distribution index k), and the statistical parameter being estimated. The magnitude of sampling error is related to sample size and variance (Fowler and Witter 1982) and can affect the number of correct classifications from sequential decision plans. In our study, sample estimators describing the unimpacted site were subject to sampling error because each data set was composed of 15 sample units, which is a small sample size compared with those recommended by statisticians but larger than usually found in benthic monitoring studies. The precision of the baseline data is not a variable in the sequential decision plan formulae. We compensated for this by defining the no-impact threshold as the mean adjusted for sampling error (i.e. $\bar{X} - 2\text{SE}$). This maintained initial classification error rates of 5% or less.

The second problem involves detecting spatial and temporal variation that are not the result of the impact event. Even in the absence of impact, benthic populations and communities will vary among sites and change dramatically between seasons or years. Only after these sources of variation are quantitatively documented and incorporated into definitions of unimpacted and impacted conditions can development-induced changes in environmental quality be indentified (Stewart-Oaten et al. 1986; Resh et al. 1988). In studies of pestiferous aquatic insects or rare and endangered species, sampling programs that use sequential decision plans may not have to adjust for natural variability; high or low population densities are important, whatever the cause.

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Appendix

Negative Binomial Distribution

Sequential decision plan, OC curve, and ASN curve formulae for the negative binomial distribution are from Waters (1955). First, the *y*-intercepts (h_1,h_2) and slope (b) of the decision lines are calculated using the following formulae:

$$h_1 = \frac{\log \dfrac{\beta}{1-\alpha}}{\log \dfrac{p_2 q_1}{p_1 q_2}} \; ; \; h_2 = \frac{\log \dfrac{1-\beta}{\alpha}}{\log \dfrac{p_2 q_1}{p_1 q_2}} \; ; \; b = k \cdot \frac{\log \dfrac{q_2}{q_1}}{\log \dfrac{p_2 q_1}{p_1 q_2}}$$

where $p_1 = m_1/k$, $q_1 = 1 + p_1$, $p_2 = m_2/k$, and $q_2 = 1 + p_2$, m_1 is the classification threshold for the lower classification category, m_2 is the classification threshold for the upper classification category, m_1 is always less than m_2, k is the distribution index, and α and β are risks of misclassification. The equation for the lower decision line is $d_1 = b(n) + h_1$ and the upper decision line is $d_2 = b(n) + h_2$ where d is the cumulative total when n sample units have been examined.

Values for the OC and ASN curves are calculated using various values of x, a "dummy variable." We used values of x ranging from 10.0 and -10.0. The OC curve is created by plotting $L(m)$ (the probability of accepting H_1) versus various means (m) where

$$L(m) = \frac{A^x - 1}{A^x - B^x} \quad \text{with } A = \frac{1-\beta}{\alpha} \; ; \; B = \frac{\beta}{1-\alpha}$$

$$m = kp \text{ with } p = \frac{1 - \left(\dfrac{q_1}{q_2}\right)^x}{\left(\dfrac{p_2 q_1}{p_1 q_2}\right)^x - 1}.$$

The ASN curve is created by plotting $E(n)$ versus various means (m) where

$$E(n) = \frac{h_2 + (h_1 - h_2)\, L(m)}{m - b}.$$

Poisson Distribution

Sequential decision plan, OC curve, and ASN curve formulae for the Poisson distribution are modified from Onsager (1976). For calculating decision lines:

$$h_1 = \frac{-\log \dfrac{1-\alpha}{\beta}}{\log \dfrac{m_2}{m_1}} \; ; \; h_2 = \frac{\log \dfrac{1-\beta}{\alpha}}{\log \dfrac{m_2}{m_1}} \; ; \; b = \frac{0.4343\,(m_2 - m_1)}{\log \dfrac{m_2}{m_1}}.$$

The equation for the lower decision line is $d_1 = b(n) + h_1$ and the upper decision line is $d_2 = b(n) + h_2$.
For calculating OC and ASN curves:

m	$L(m)$	$E(n)$
0	1	$h_1/-b$
m_1	$1-a$	General formula
b	$h_2/(h_2 - h_1)$	$(h_1)\,(h_2)/-b$
m_2	β	General formula

The general formula for $E(n)$ is

$$E(n) = \frac{L(m)\,(h_1 - h_1) + h_2}{m - b}.$$

Normal Distribution

Sequential decision plan, OC curve, and ASN curve formulae for the normal distribution are from Waters (1955). For calculating decision lines:

$$h_1 = \frac{s^2 \cdot \ln \dfrac{1-\alpha}{\beta}}{m_2 - m_1} \; ; \; h_2 = \frac{s^2 \cdot \ln \dfrac{1-\beta}{\alpha}}{m_2 - m_1} \; ; \; b = \frac{m_1 + m_2}{2}$$

where s^2 is the sample variance. The equation for the lower decision line is $d_1 = b(n) - h_1$ and the upper decision line is $d_2 = b(n) + h_2$. For calculating the OC and ASN curves:

$$L(m) = \frac{(e)^{t_1} - 1}{(e)^{t_2} - 1} \quad \text{and } E(n) = \frac{L(m)\,(h_1 + h_2) - h_1}{m - b}$$

where $t_1 = h_1 K$, $t_2 = (h_1 + h_2)K$, $K = 2(b-m)/s^2$, and $e = 2.71828$. Computer programs and a manual that describes the preparation of sequential decision plans in detail are available from the authors.

Influence of pH on the Acute Lethality of Fenitrothion, 2,4-D, and Aminocarb and Some pH-Altered Sublethal Effects of Aminocarb on Rainbow Trout (*Salmo gairdneri*)

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Doe, K. G., W. R. Ernst, W. R. Parker, G. R. J. Julien, and P. A. Hennigar. 1988. Influence of pH on the acute lethality of fenitrothion, 2,4-D, and aminocarb and some pH-altered sublethal effects of aminocarb on rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 45: 287–293.

Three pesticides, fenitrothion, 2,4-D, and aminocarb, were tested in static 96-h acute lethal toxicity tests using fingerling rainbow trout (*Salmo gairdneri*) at pH 4.6, 5.6, 6.9, and 8.5. The toxicity of aminocarb, a base, increased significantly with increasing pH. Conversely, the toxicity of the acidic pesticide 2,4-D increased with decreasing pH. The toxicity of the neutral pesticide fenitrothion did not change significantly with changing pH. Subsequent tests were performed on trout fingerlings with aminocarb to determine the effect of two exposure pH's on brain acetylcholinesterase activity and whole-body aminocarb residue. Brain acetylcholinesterase was found to be inversely proportional to whole-body aminocarb content of fish. In fish exposed at pH 4.6, brain acetylcholinesterase was maximally depressed at 6 h, after which it recovered to within the control range. Whole-body aminocarb concentrations rose to a maximum within 6 h and subsequently declined to low levels. In fish exposed at pH 8.2, brain acetylcholinesterase dropped below the control range by 1 h and remained low until all fish died by 72 h. A maximum whole-body aminocarb concentration was reached within 1 h and remained elevated until the fish died. Several explanations for the observed results are presented.

Trois pesticides, soit le fénitrothion, le 2,4-D et l'aminocarbe ont fait l'objet d'expériences statiques de 96 h afin d'en déterminer la toxicité létale aiguë pour des tacons O+ de truite arc-en-ciel (*Salmo gairdneri*) aux pH 4,6, 5,6, 6,9 et 8,5. La toxicité de l'aminocarbe, une base, a nettement augmenté en fonction d'une augmentation du pH. Inversement, la toxicité du 2,4-D, un pesticide acide, a augmenté en fonction d'une baisse du pH tandis que la toxicité du fénitrothion, un pesticide neutre, n'a pas varié de façon significative en fonction d'une variation du pH. On a par la suite exposé des tacons O+ à de l'aminocarbe afin de déterminer l'incidence de deux pH sur l'activité céphalique de l'acétylcholinestérase et sur la concentration de résidus d'aminocarbe dans tout le corps. L'activité céphalique de l'acétylcholinestérase était inversement proportionnelle à la teneur totale en aminocarbe. Chez les poissons exposés à un pH 4,6, l'activité de l'acétylcholinestérase a atteint son minimum après 6 h d'exposition après quoi elle est revenue à un niveau se situant dans l'écart témoin. Les concentrations totales d'aminocarbe ont atteint un maximum en dedans de 6 h et ont par la suite baissé à de faibles niveaux. Chez les poissons exposés à un pH 8,2, l'activité céphalique de l'acétylcholinestérase a chuté sous le niveau témoin après 1 h d'exposition et est demeurée faible jusqu'à la mort de tous les poissons après 72 h. Une concentration totale maximum d'aminocarbe a été atteinte en dedans de 1 h; elle est demeurée élevée jusqu'à la mort des poissons. On présente plusieurs explications des résultats observés.

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Annually, there are large-scale forestry insecticide and herbicide spray programs conducted in the Maritime Provinces of Canada. The most commonly used chemical insecticides in this region are fenitrothion (*O,O*-dimethyl *O*-(4-nitro-*m*-tolyl)phosphorothioate) and aminocarb (*p*-dimethylamino-*o*-*m*-tolyl *N*-methylcarbamate). The herbicide 2,4-D ((2,4-dichlorophenoxy)acetic acid) has seen extensive use in the control of unwanted vegetation. Large-scale spraying of pesticides results in the deposit of spray formulations in nontarget environments such as adjacent aquatic systems (Eidt and Sundaram 1975; Norris 1981). Excluding accidental releases, the operational dosage rates of these pesticides are not expected to cause direct lethal responses in exposed native fish populations (Symons 1977; NRCC 1978; Martens et al. 1980).

Water quality characteristics are known to modify the toxic potential of organic chemicals in general (Fry 1971) and specifically pesticides (Woodward and Mauck 1980; Woodward and Mayer 1978). A water quality characteristic that is changing notably in certain surface waters within this region is pH (Kelso et al. 1986). While the effects of ambient water pH changes alone on aquatic fauna have been well documented (McDonald 1982), the effects of pH in concert with organic contaminants have not been well explored. An experiment was therefore

TABLE 1. Characteristics of dilution water used in toxicity experiments. Hardness as $CaCO_3$; ND = not detected.

Parameter	Mean ± SD or range
Hardness (mg/L)	21.6 ± 2.4
Conductivity (mmho/cm)	0.067 ± 0.002
Turbidity (JTU[a])	0.32 ± 0.8
Alkalinity (mg/L)	7.6 ± 2.5
Magnesium (mg/L)	0.55 ± 0.04
Sulphate (mg/L)	6.6 ± 0.5
Sodium (mg/L)	3.9 ± 0.8
Calcium (mg/L)	7.7 ± 0.7
Arsenic (mg/L)	<0.05
Cadmium (mg/L)	<0.01
Chromium (mg/L)	<0.01
Copper (mg/L)	<0.01
Iron (mg/L)	0.09 ± 0.03
Lead (mg/L)	<0.02 – 0.02
Zinc (mg/L)	<0.01
Nickel (mg/L)	<0.01
Manganese (mg/L)	<0.01 – 0.04
Aluminum (mg/L)	<0.025 – 0.18
Chloride (mg/L)	8.1 ± 0.4
Fluoride (mg/L)	0.9 ± 0.2
Potassium (mg/L)	<0.25 – 0.51
Total carbon (mg/L)	6.6 ± 1.3
Chlorine (μg/L)	ND – 70[b]

[a]Johnson turbidity units.

[b]Measured daily on water supply; 89% of values less than or equal to 35 μg/L.

undertaken to determine the effect of different pH levels on the acute lethality of fenitrothion, 2,4-D, and aminocarb to rainbow trout (*Salmo gairdneri*) and to elucidate the mechanism by which changing pH alters pesticide toxicity.

Methods and Materials

Toxicity Tests

Static 96-h lethal toxicity tests were performed on fingerling rainbow trout. Average wet weights of fish in each test ranged from 3.5 ± 1.3 (± SD) to 9.1 ± 3.7 g. Test volume was 40 L and there were 10 fish per test tank, which were aerated at a rate of 200–300 mL/min and were lined with new 102-μm-thick polyethylene liners for each test. During the tests, temperature and dissolved oxygen were measured daily in each tank and ranged from 13.5 to 16.5°C and 7.1 to 10.6 mg/L, respectively.

Fish were held in continuously flowing dechlorinated Dartmouth municipal water at 15 ± 2°C and fed a commercial trout pellet at a rate of 3% of their body weight per day from 1 to 5 wk before testing. Fish were not fed for approximately 24 h before a test was started. The photoperiod for holding and testing was 12 h light : 12 h dark. Water used to hold the fish was also used for dilution and as control water during the toxicity tests. This water had been charcoal filtered and dechlorinated by ultraviolet light. It was soft and had low levels of heavy metals (Table 1). To remove any last traces of chlorine in the water, it was added to the test tanks and aerated at least 24 h before a test was started.

The pesticides used in this study were technical grades of fenitrothion, 2,4-D, and aminocarb. Fenitrothion and 2,4-D were blended with water for 1 min then added to the test tank and stirred by hand for 1 min. For aminocarb, a stock solution was made up in acetone and appropriate volumes were added to the test tanks and stirred in vigorously. The maximum acetone concentration in a test tank was 471 mg/L, a concentration that is much less than the 96-h LC50 of 5500 mg/L (EP Atlantic Region, unpubl. data). At 0 and 96 h, selected test solutions were sampled for chemical analysis of the pesticide being tested.

Tests with each pesticide were run at an average measured pH of 4.6, 5.6, 6.9, and 8.5 (Table 2). Control tanks were also adjusted to these pH values. The pH of each test solution was measured twice or more daily and adjustments were made by adding 0.5 *M* H_2SO_4 or 1*M* NaOH. For the pH 8.5 test solution, 1*M* NaOH and 0.5 *M* H_3BO_3 were used, usually in a 1:2 ratio.

Acute lethal toxicity was expressed as 96-h LC50 (median lethal concentration) derived by probit analysis using the BMD03S program (Dixon 1973) where two or more test concentrations experienced partial mortalities of the fish populations. Where there were fewer than two partial mortalities of fish populations in any single LC50 test, the LC50s were calculated using the binomial test method (Stephan 1977). Because of the limited number of chemical analyses conducted on the test solutions, it was decided to express test results based on nominal test concentrations.

Aminocarb Uptake and Brain Acetylcholinesterase Inhibition

Test fish were fingerling rainbow trout of average weight 73.7 ± 18.1 g and were held as described previously. Tests were static, of 96-h duration, and conducted in refrigerated fiberglass tanks lined with clean 102-μm polyethylene and containing 300 L of solution. Aeration was provided at a rate of 1 L/min. Temperature and dissolved oxygen were measured daily in each tank and ranged from 12 to 18°C and 7.4 to 10.2 mg/L, respectively. The pH of the test solutions was adjusted as described previously and averaged 4.6 (range 4.1–6.2) and 8.2 (range 7.0–8.7).

Fish were exposed to 10 mg aminocarb/L or to control/dilution water at pH 4.6 and pH 8.2. Acetone was used as a carrier–solvent as in the acute toxicity tests. Five fish were removed from each test tank for brain acetylcholinesterase assays and aminocarb concentration determination in tissues at 0, 1, 3, 6, 25, 52.5, 72, and 96 h or until total mortality occurred. Time zero samples were obtained by placing fish in the exposure tanks and immediately removing them.

Fish were killed by cervical dislocation and rinsed with distilled deionized water followed by pesticide-grade acetone and pesticide-grade chloroform. Wash bottles were used to ensure complete surface cleaning. Fish for tissue analyses were then frozen. Samples usually consisted of a pool of four fish, but two fish were used towards the end of the experiments. Fish for brain acetylcholinesterase assays were rinsed with water only, heads were removed, wrapped in aluminum foil, placed in plastic "whirl pak" bags, and stored at −20°C until analyzed for brain acetylcholinesterase activity. Samples were stored for no longer than 6 mo before cholinesterase analyses were performed.

Water samples were removed from the nominal 10 mg aminocarb/L exposure tanks at 1 h and at termination (96 h or when total mortality had occurred, whichever was sooner) for chemical analysis. At 1 h, 10 mg aminocarb/L was measured at pH 4.6, while 11 mg aminocarb/L was measured at pH 8.2. By termination, 9.5 mg/L was measured at pH 4.6, while 8.4 mg/L was measured at pH 8.2.

TABLE 2. Measured pH (mean ± SD) of toxicity test solutions.

Pesticide	Nominal pH 4.5	5.5	7.0	8.5
Fenitrothion	4.63 ± 0.34	5.63 ± 0.23	6.88 ± 0.27	8.49 ± 0.32
2,4-D	4.54 ± 0.24	5.6 ± 0.30	6.8 ± 0.35	8.48 ± 0.32
Aminocarb	4.56 ± 0.22	5.64 ± 0.18	6.89 ± 0.23	8.4 ± 0.41

Cholinesterase Assays

Immediately prior to analyses, heads were thawed, dissected, and whole brains removed. Three brains were pooled, weighed, and assayed spectrophotometrically for acetylcholinesterase activity using the method of Hill and Flemming (1982). Specific activity is expressed as micromoles of acetylcholine hydrolyzed per gram of brain tissue per minute.

Chemical Analytical Methodology

The analysis of fenitrothion and aminocarb was carried out according to methods previously described (Department of Environment 1979). Briefly, water samples containing fenitrothion were adjusted to pH 7 and extracted with hexane. The solvent was concentrated and analyzed by gas chromatography coupled with flame photometric detection. Water samples containing aminocarb were adjusted to pH 7 and extracted with chloroform. The chloroform was exchanged with benzene prior to analysis by gas chromatography and nitrogen–phosphorus detection. Whole fish tissue containing aminocarb was homogenized with acetonitrile. Aminocarb was extracted from an acetonitrile and water mixture with benzene and analyzed in the same manner as water samples. Quantitative recoveries of 2,4-D in water samples were obtained based on a method for phenoxy acid herbicides described previously (Inland Waters Directorate 1979) with the following modifications: benzene replaced chloroform as the extraction solvent; diazomethane derivitization was substituted for boron trifluoride derivitization; and electron capture detection was replaced with Hall electrolytic conductivity detection in the chloride mode (GC conditions: column temperature, 190°C; carrier gas He, 50 mL/min, H_2, 50 mL/min; furnace temperature, 925°C; electrolyte, 50% *n*-propanol–water at 1 mL/min).

Results

Toxicity Tests

The three pesticides behaved differently when the test solution pH was varied (Fig. 1). Fenitrothion toxicity appeared to be very similar at all four pH levels tested. However, the LC50 at pH 4.6 was slightly lower than the LC50 at the other pH values. As pH increased, the toxicity of 2,4-D decreased dramatically. As pH 8.5, the 96-h LC50 was more than an order of magnitude higher than at pH 4.5. There was no indication of stress on the fish at 1000 mg 2,4-D/L at pH 8.5, even after 6 d of exposure. The effect of changing pH on aminocarb toxicity was the inverse of 2,4-D toxicity. At pH 8.4 the 96-h LC50 of aminocarb was more than an order of magnitude less than at pH 4.6. The repeatability of the aminocarb toxicity tests was also very good, as evidenced by the fact that for three tests at one pH (7.0) conducted on two separate test dates and with two separate fish batches, the 96-h LC50s were 56, 49, and 60 mg/L, with a coefficient of variation of 10.1%. There were no control mortalities in any of the tests conducted.

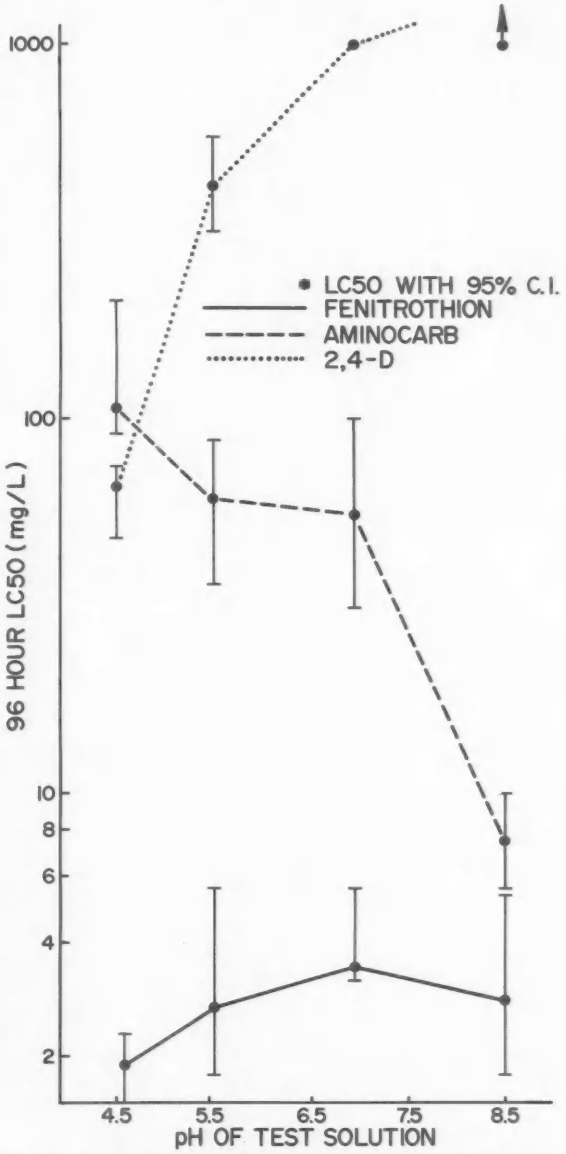

FIG. 1. Acute toxicity (96-h LC50) of fenitrothion, aminocarb, and 2,4-D to rainbow trout exposed at various pH levels. Verticle bars represent 95% confidence intervals for the calculated LC50 values.

Some dead fish in fenitrothion exposure tanks developed distended abdomens, and upon dissection, the stomach and intestinal tract of fish were found to be swollen by a clear fluid containing some food particles. A similar observation has been

noted by Klaverkamp et al. (1977). Since condition factors ($W/L^3 \times 100$) for all fish surviving the exposures were not significantly different from the condition factors of control fish, the phenomenon apparently occurs at or near death.

At pH 8.5, the aminocarb test solutions turned from clear to pink as early as 19 h into the test. This eventually turned to a deep wine-red colour. This colour change did not occur with other pesticides or at other pH values. When these test solutions were restocked with fish at pH 8.5, 1 wk after the original test started, there was no difference in toxicity. After 2 wk at pH 8.5, and with the test solutions wine-red in colour, the pH was adjusted to 6.9 and the solutions were again restocked. The 96-h LC50 was now 56.6 mg/L, identical to the 96-h LC50 of a freshly prepared aminocarb test solution at the same pH. There was thus no loss in toxicity after 2 wk of aeration at pH 8.5, and the observed increased toxicity as this pH was reversible when test solution pH was lowered.

Fenitrothion concentrations appeared to decrease to about one half the initial measured concentration after 4 d at all pH levels tested (Table 3). Concentrations of 2,4-D remained stable at pH 4.5 and 6.8 but decreased by about 20% of the initial measured values at pH 8.5 at the end of the test period. Aminocarb concentrations remained steady at pH 6.9 and 8.4 but showed a reduction of about 30–50% in the pH 4.6 tests over the 96-h test period.

Aminocarb Uptake and Brain Acetylcholinesterase Inhibition

Results of fish tissue analysis for aminocarb content and brain cholinesterase assays of fish from the 10 mg/L exposure concentration at pH 4.6 and 8.2 are presented in Fig. 2a and 2b, respectively. At pH 4.6 (Fig. 2a), the aminocarb residue concentrations in fish tissues increased for the first 6 h to a high of 9.1 μg/g, after which it declined to a low concentration of 1 μg/g at 25 h and remained at about that level for the duration of the exposure. Fish became sluggish, but recovered by 72 h. The aminocarb bioconcentration factor (measured concentration in fish divided by measured concentration in water) for fish exposed to pH 4.6 at 96 h was approximately 0.1.

In the fish exposed at pH 8.2 (Fig. 2b), whole-body aminocarb content rose to a slightly higher level much more rapidly than in fish exposed to pH 4.6, and concentrations remained elevated until the fish died at 72 h. Fish became progressively more sluggish and lost equilibrium as exposure time increased. The bioconcentration factor in fish exposed at pH 8.2 at 72 h was approximately 1.7.

Control brain acetylcholinesterase activity was variable, and the most meaningful acetylcholinesterase activity values from treated fish are those which fall outside the range of control values. In the fish from the treatments of 10 mg aminocarb/L at pH 4.6 and 8.2 the brain acetylcholinesterase activity was inversely proportional to the aminocarb content of whole tissues. The enzyme activity of fish exposed at pH 4.6 was maximally depressed at 6 h, after which they recovered to control values. The brain enzyme activity in fish exposed at the higher pH, on the other hand, was maximally depressed by 3 h and remained depressed below the range of control values for the duration of the exposure.

Discussion

The pH of test solutions, with the exception of the lowest, probably had little direct physiological effect on exposed fish (McDonald 1982). The lowest exposure pH (4.6) was below the threshold for salmonid gill injury of between pH 5.6 and 5.2 observed by Daye and Garside (1976). The results of other studies have suggested that gill changes (Lloyd and Jordan 1964) and osmoregulatory changes (McDonald et al. 1983) are relatively minor and reversible at exposure pH of greater than 4.5. The fact that fenitrothion toxicity changed little over the range of pH exposures tested in our study is at least indirect evidence that pH damage to fish was not an important factor in modifying the toxic effects of pesticides.

The LC50 values obtained at neutral pH levels during these tests are in reasonably close agreement to those previously

TABLE 3. Results of chemical analyses on selected test solutions during the acute lethal toxicity tests.

Pesticide	Nominal pH	Nominal concn. (mg/L)	Initial measured concn. (mg/L)	Final measured concn. (mg/L)[a]	% reduction in measured concn. (mg/L)
Fenitrothion	4.6	1.0	0.96	0.43	55
		10.0	9.2	4.3	53
	6.9	1.0	1.0	0.48	52
		10.0	8.7	3.3	63
	8.5	1.0	1.0	0.53	47
		10.0	6.1	4.7	23
2,4-D	4.5	32.0	22.7	24	−6
		320.0	236.0	240.0	−3
	6.8	180.0	78.1	140.0	−79
		1000.0	858.0	910.0	−6
	8.5	100.0	75.6	59.0	21
		1000.0	670.0	440.0	34
Aminocarb	4.6	56.0	60.0	38.0	37
		180.0	169.0	75.0	56
	6.9	10.0	8.4	8.8	−5
		100.0	84.0	85.0	−5
	8.4	10.0	9.1	6.9	24
		100.0	77.0	82.0	−6

[a]Final samples for chemical residue analyses taken at termination of tests (96 h).

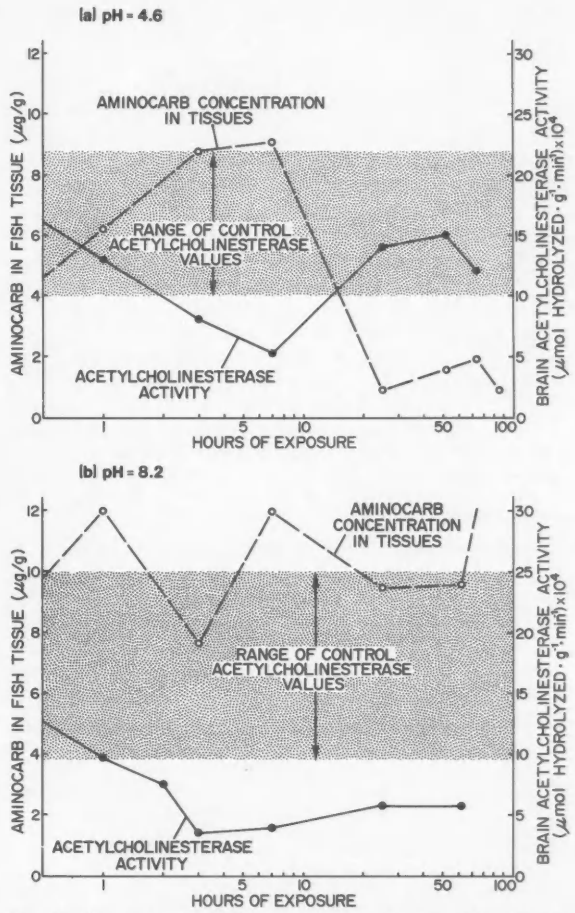
(a) pH = 4.6
AMINOCARB CONCENTRATION IN TISSUES
RANGE OF CONTROL ACETYLCHOLINESTERASE VALUES
ACETYLCHOLINESTERASE ACTIVITY
AMINOCARB IN FISH TISSUE (μg/g)
BRAIN ACETYLCHOLINESTERASE ACTIVITY (μmol HYDROLYZED · g⁻¹·min⁻¹)×10⁴
HOURS OF EXPOSURE
(b) pH = 8.2
AMINOCARB CONCENTRATION IN TISSUES
RANGE OF CONTROL ACETYLCHOLINESTERASE VALUES
ACETYLCHOLINESTERASE ACTIVITY
AMINOCARB IN FISH TISSUE (μg/g)
BRAIN ACETYLCHOLINESTERASE ACTIVITY (μmol HYDROLYZED · g⁻¹·min⁻¹)×10⁴
HOURS OF EXPOSURE

FIG. 2. Measured whole-body concentration of aminocarb and brain acetylcholinesterase activity of rainbow trout exposed to 10 mg aminocarb/L at (a) pH 4.6 and (b) pH 8.2.

reported for salmonids exposed to fenitrothion (Klaverkamp et al. 1977; Ernst et al. 1980; Sanders et al. 1983), 2,4-D (Meehan et al. 1974; Martens et al. 1980), and aminocarb (Woodward and Mauck 1980; Sanders et al. 1983).

The fact that higher pH decreased the toxicity of 2,4-D, increased the toxicity of aminocarb, and did not change the toxicity of fenitrothion has several possible explanations. These might include rapid pH-mediated changes in toxicants that change their availability to the test animals or physiological changes in the test animals themselves that made them more or less susceptible to the toxicants.

The approximately 50% reduction in fenitrothion in the test solutions at all pHs is consistent with the findings of Sundaram (1973), who found 50% disappearance of fenitrothion after 20 h in a pH range of 5.4–8.2. The observed decrease could be due to hydrolysis, photodecomposition (e.g. Miyamoto 1977), or some other process.

Measurement of 2,4-D showed essentially no reduction over time at pH 4.6 and 6.9. It has been well established that 2,4-D is much more stable to hydrolysis at lower pH levels (Zepp et al. 1975), and so hydrolysis may account for the reduction in 2,4-D concentration at pH 8.5 and some of the reduced toxicity at higher pH levels. Aminocarb has also been previously observed to be more stable at lower pH levels (NRCC 1982; Weinberger and Greenhalgh 1983); however, the results of our limited chemical analyses would seem to contradict this. The increased toxicity of aminocarb at higher pH levels cannot be explained on the basis of the established aminocarb hydrolytic rates, unless hydrolytic breakdown products produced as pH increases are more toxic than the parent compound, as was found for mexacarbamate (Mauck et al. 1977).

The changes of colour of test solutions which accompanied pH changes have previously been noted by Vassilieff and Ecobichon (1982); however, unlike our study, these investigators noted that the colour changes were reversible and test solutions lost their potency rapidly. Recently, studies have indicated that the colour in aminocarb solutions is due to the formation of highly coloured methyl paraquinone (V. Mallet, Department of Chemistry, University of Moncton, N.B.) which would probably not be reversible. This would seem to support our observations.

The results of chemical analyses of test solutions indicate that toxicity changes with changing pH may be partly explained by different pesticide hydrolysis rates in the case of 2,4-D; however, the change in aminocarb toxicity cannot convincingly be explained in this way.

A more comprehensive and satisfying explanation for the toxicity changes of the various pesticides with pH changes is contained in the pH-partition theory (e.g. Jollow and Brodie 1972), which states that undissociated organic molecules pass across biological membranes more readily than do ionized forms of the same chemical. Since toxicity is generally a consequence of the amount of toxicant which is available to target sites, increased absorption and therefore toxicity is a result of a change in pH which increases the proportion of the undissociated form.

Fenitrothion does not ionize and it should therefore cross biological membranes at a constant rate, regardless of the pH of the toxicant solution. The results of the fenitrothion toxicity tests agree well with this theory. An acidic pesticide such as 2,4-D, on the other hand, dissociates to a greater extent as the pH of the solution increases, although not linearly (Fig. 3), and therefore the toxicity should be reduced by such a change in pH. Our results indicate that 2,4-D toxicity decreased with increasing pH and the decrease in toxicity was proportional to the amount dissociated. The relationship was not linear over the ranges tested, indicating that other factors are involved in modifying toxicity, such as hydrolysis discussed earlier. Woodward and Meyer (1978) found that water pH did not affect the toxicity of three esters of 2,4-D. Because these esters do not ionize and since they do not readily degrade to ionizable forms (NRCC 1978), they would behave in a similar manner to fenitrothion and so their toxicity would not be influenced by water pH. As expected from the theory, the toxicity of aminocarb, which is a basic pesticide that dissociates to a greater extent as the pH of the solution decreases (Fig. 3), decreased substantially with a decrease in pH.

The results of both the aminocarb uptake measurements and cholinesterase assays indicate that after 10 h of exposure there is a greater amount of pesticide within fish exposed at the higher pH, and this caused a more pronounced physiological effect as measured by brain cholinesterase inhibition. These effects are even more pronounced after 10 h of exposure. This may be taken as further evidence that it is the amount of undissociated

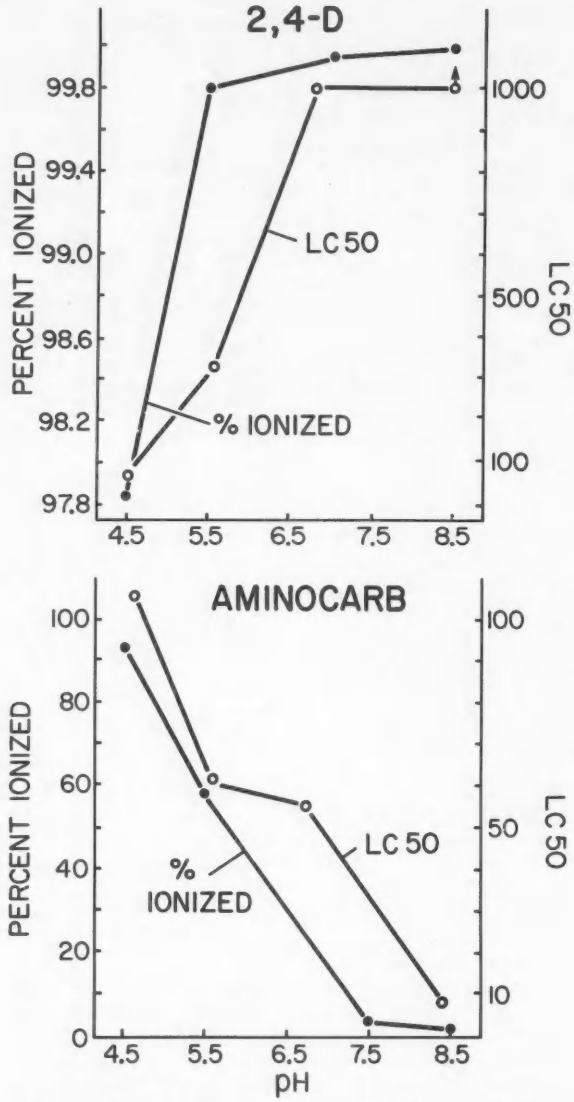

FIG. 3. Toxicity and percent of un-ionized 2,4-D and aminocarb at various pH levels.

pesticide which is in the exposure solution that is important in causing toxic effects.

It is also particularly noteworthy that the aminocarb content of the fish exposed at the high pH remained elevated for the duration of exposure, while the aminocarb content of the fish exposed at low pH returned to low levels after approximately 10 h of exposure. It must be presumed that pesticide elimination or metabolic rates were adequate to cope with pesticide uptake in the fish exposed at the lower pH, but not in fish at the higher pH.

In general, a cholinesterase depression of greater than 50% of control value has been associated with some degree of lethality (Macek et al. 1972; Jarvinen et al. 1983) and depressions of as little as 10% have been associated with sublethal effects (Jarvinen et al. 1983). In this study, brain acetylcholinesterase activity was maximally decreased to 42% of mean control fish values at pH 4.6 and maximally depressed to 25% of mean control values in the pH 8.2 exposed fish. In addition, dead fish from the pH 8.2 exposed tanks had cholinesterase activity levels which were depressed 92% below mean control values. Since mortality occurred in the pH 8.2 test group, these results would seem to indicate that individual mortalities may occur when some cholinesterase values in the population are depressed to 25% of mean control values.

In summary, the study demonstrates that the pH of exposure solutions significantly modifies acute pesticide toxicity to fish. This indicates the need for closer evaluation of the relevance of laboratory generated toxicity information as it pertains to field exposure, particularly in light of the declining pH of some surface waters. The lowest pH used in these tests (4.6) very closely approximates the pH of many rivers in eastern Canada (Watt et al. 1983), and the results, therefore, are environmentally relevant. Regulatory agencies that are responsible for evaluating pesticide behaviour prior to registration should take this factor into account.

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Relationships Between Concentrations of Sedimentary Variables and Trophic State in Florida Lakes

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Brenner, M., and M. W. Binford. 1988. Relationships between concentrations of sedimentary variables and trophic state in Florida lakes. Can. J. Fish. Aquat. Sci. 45: 294–300.

Midlake sediment samples from 97 Florida lakes were analyzed for organic matter (LOI 550°C), carbonate (LOI 550–990°C), total C, N, and P, and pigment ($SCDU_{665}$) content. We used simple correlation and multiple regression to examine relationships between concentrations of selected sediment constituents, or their ratios, and trophic state (Carlson's TSI) of the overlying waters. Simple concentrations of sediment variables are poor predictors of TSI. The best simple correlation relates N per gram of organic matter to TSI–Chl *a* but explains only 27% of the variance in trophic state. The best multiple regression model incorporates five independent sediment variables and accounts for 43% of the variance in TSI–Chl *a*. Reasons for the poor relationships include (1) interlake variation in the dilution of organic matter and pigments by allochthonous inorganics, (2) variation among lakes in the diagenesis of surface sediments, (3) differential contributions of insoluble, large particulate, inorganic P, and (4) failure of open-water TSI measures to account for contributions to sediments from littoral and allochthonous sources.

On a analysé des échantillons de sédiment prélevés dans les eaux centrales de 97 lacs de la Floride afin d'en déterminer les teneurs en matière organique (LOI 550°C), en carbonate (LOI 550–990°C), en C, N et P total et en pigment ($SCDU_{665}$). Les relations entre, d'une part, les concentrations de certains constituants du sédiment ou leur quotient et, d'autre part, l'état trophique (TSI de Carlson) des eaux surjacentes ont été examinées à l'aide d'analyses simples de corrélations et de régressions multiples. On a ainsi découvert que les concentrations simples des paramètres du sédiment sont de pauvres prédicteurs du TSI. La meilleure corrélation simple établit un lien entre N par gramme de matière organique et le TSI–Chl *a*, mais n'explique que 27 % de la variance de l'état trophique. Le meilleur modèle de régression multiple réunit cinq paramètres indépendants du sédiment et explique 43 % de la variance du TSI–Chl *a*. Ces relations médiocres sont le résultat des facteurs suivants : 1) une variation inter-lacustre de la dilution de la matière organique et des pigments par des matières inorganiques allochtones, 2) une variation inter-lacustre de la diagenèse des sédiments superficiels, 3) un apport différentiel de grosses particules insolubles de P inorganique, et 4) le fait que les quantifications du TSI en eau libre ne tiennent pas compte des apports sédimentaires de sources littorale et allochtone.

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Several studies have shown that there is a correlation between concentrations of variables in lake sediments and characteristics of the overlying waters. Prior to sewage diversion from Lake Washington, sedimentary P concentrations tracked increasing amounts of water-column P through time (Shapiro et al. 1971; Edmondson 1974). In a long-term study of Lake Washington, P loss to sediments was positively correlated with both P supply to the lake and water concentration of the element (Edmondson and Lehman 1981). For a group of Polish lakes, Rybak (1969) found that organic matter content of surface sediments increased as a function of trophic state. In the English Lakes District, concentrations of sedimentary pigments and sulfur were shown to provide an accurate index of primary productivity (Gorham et al. 1974).

Across a suite of lakes representing a trophic gradient, a positive correlation exists between P supply, P concentration in the water, and P loss to the sediments (Vollenweider 1968, 1975; Dillon and Rigler 1974; Shannon and Brezonik 1972; Reckhow and Chapra 1983).

For 15 Florida lakes studied by Flannery et al. (1982), percent organic matter in surficial muds was positively correlated with trophic state ($r = 0.59$, $P < 0.05$). Surface sediment C:N ratios were negatively correlated with ln Chl *a* in the water column ($r = -0.85$, $P < 0.01$), and a weak positive correlation was found between sedimentary chlorophyll derivatives, expressed per gram of organic matter, and water column Chl *a* ($r = 0.48$, $P = 0.07$).

Little has been reported on the sediments of Florida lakes, and in this paper we expand on the work of Flannery et al.

(1982) and report surface sediment data (weight loss on ignition (LOI) 550°C, LOI 550–990°C, total C, N, P, and pigments ($SCDU_{665}$)) for 97 Florida lakes. Chlorophyll *a* data are available for 91 of the study lakes and show a considerable concentration range (1.0–122.6 mg Chl $a \cdot m^{-3}$). Sediment and water-column data from these 91 lakes were used to explore the relationship between concentrations of sediment variables and trophic state of the overlying waters.

Methods

Lake Selection

We selected 97 lakes for our sediment study. The lakes were chosen from a large, limnological data set compiled and maintained by the Water Resources Research Center at the University of Florida, Gainesville, FL. The limnological information is summarized in Huber et al. (1982). Nonrandom selection of lakes was based on several criteria: (1) lakes were chosen to reflect a wide range of trophic states, (2) lakes were selected over a broad geographic range, and (3) lakes were picked based on accessibility.

The 97 chosen lakes vary greatly with respect to several limnological variables (e.g. pH, alkalinity, conductivity). Limnological data presented in Table 1 are medians and generally summarize several years of data. We chose medians to reduce the effect of extreme values. Eighty percent of the limnological data entries were gathered since 1970.

We included both large and small lakes in our survey. Surface area is reported for 92 of the lakes we examined and ranges from 7 to 14 143 ha. Like most Florida lakes, our study basins were typically shallow. Huber et al. (1982) reported maximum depths for 98 lakes, half of which have values ≤4 m. In our data set, maximum depth is reported for 30 lakes, of which 15 are ≤4 m deep. Only three of the 30 lakes exceed 10 m of depth.

The majority of Florida lakes are "collapse" or solution basins in limestone terrain (Stubbs 1940), a factor that may explain the popular misconception that most of Florida's warm temperate and subtropical lakes are hardwater, eutrophic systems. Many of the lakes are isolated from deep groundwater and contain relatively soft water (see Table 1). Open-water Chl *a* concentrations reflect oligomesotrophic conditions in the majority of Florida lakes. For 305 lakes reported by Huber et al. (1982), 73% had less than 20 mg Chl $a \cdot m^{-3}$. Among our 91 lakes with reported Chl *a* values, 54 possess <10 $mg \cdot m^{-3}$ and 15 fall in the range from 10 to 20 $mg \cdot m^{-3}$ (Table 1).

Sediments

Each study lake was visited once in the summer of 1982 and an Ekman grab was taken near the center of the lake. Only undisturbed, surficial samples were kept. The top 2 cm of mud was removed from the dredge and subsamples from the grab were stored in jars for later analyses. ^{210}Pb dates from cores taken in 20 Florida lakes of variable trophic states indicate that the surface mud samples represent 2–10 yr of sediment accumulation (M. W. Binford, unpubl. data).

Water, organic matter, and carbonate contents of the sediments were determined by sequential weight loss at 100, 550, and 550–990°C (Dean 1974). Total C was measured with a LECO induction furnace. Total N was measured by a modified Kjeldahl digestion (Nelson and Sommers 1972). Samples for total P were digested after ignition (Andersen 1976). Phosphorus concentrations were determined spectrophotometrically using ascorbic acid–ammonium molybdate (American Public Health Association 1975). Sedimentary pigments were extracted and measured according to the methods of Sanger and Gorham (1972). In this study, one sedimentary chlorophyll degradation unit (SCDU) is equal to an absorbance of 0.1 in a 1-cm cell when extractant volume was brought to 100 mL.

Results

Organic matter averages 39.7% of the dry bulk sediment in Florida surface muds (Table 1). The sediment organic matter content varies considerably among the lakes, ranging from 0.8 to 84.2%. Sediments from 96 of the lakes lost 0.1–4.5% of their bulk dry weight on ignition between 550 and 990°C (Table 1). Seventy-five samples lost <3.0%. The highest carbonate content was measured in spring-fed Lake Panasoffkee in which the LOI 550–990°C was 28.9%. Total C measurements in sediments of the survey lakes range over two orders of magnitude, from 4 to 476 $mg \cdot g^{-1}$ (Table 1). Total C represents a nearly constant (53 ± 5%) proportion of the organic matter (LOI 550°C) fraction.

Total N values display high variability, ranging from 0.6 to 42.4 $mg \cdot g^{-1}$ (Table 1). Total P concentrations in sediments vary among the lakes, from 0.07 to 8.09 $mg \cdot g^{-1}$. Eleven of the lakes have >3 mg $P \cdot g^{-1}$ in their surface muds, and 10 of these overlie phosphatic limestone deposits, are located in urban settings, or both. Alachua County's Cooter Pond is the exception. It is a small, shallow lake and the surrounding land is managed for a private hunting preserve.

Sedimentary pigments, expressed per gram of dry sediment, range over nearly three orders of magnitude, from 0.02 to 9.98 $SCDU \cdot g^{-1}$ (Table 1). The variability in part reflects the differing proportions of organic matter in the sediments. Nevertheless, even when pigments are expressed in terms of their concentration per gram of organic matter, there is still considerable interlake variation (0.20–16.64 $SCDU \cdot g^{-1}$).

Discussion

Relationships Among Sediment Variables

When expressed as a fraction of the dry mass, all sediment variables except total P are positively correlated with organic matter (Table 2). Across the suite of study lakes, total C is highly correlated with organic matter ($r = 0.99$). This correlation, and the low LOI 550–990°C, found in 96 lakes, suggests that most of the C in Florida muds is bound in organics. Total N is highly correlated with both organic matter content ($r = 0.93$) and total C ($r = 0.91$).

The C:N weight ratio of Florida surface sediments ranges from 6.7 to 22.0, with a mean and standard deviation of 13.0 ± 3.1. Flannery et al. (1982) reported similar C:N weight ratios ($\bar{x} = 12.1$, SD = 2.03) for their 15-lake data set. C:N ratios of surficial muds reflect differing proportions of allochthonous and autochthonous organic matter inputs to the sediment (Hutchinson 1957; Mackereth 1966) as well as between-basin differences in sediment diagenesis. The impact of lake morphometry on sediment C:N ratio was examined by simple correlation, although morphometric data were not available for all variables in all lakes. Sediment C:N ratio was uncorrelated with maximum water depth ($n = 30$), average water depth ($n = 28$), water depth of the midlake sediment sample ($n = 96$),

TABLE 1. Limnological and sedimentological characteristics for 97 Florida lakes. Water data are median values of the
represent concentrations expressed per gram of dry bulk sediment and were run on surface mud from a midlake sam

			Sediments						
	Lake	County	LOI 550°C (%)	LOI 550–990°C (%)	Total C ($mg \cdot g^{-1}$)	Total N ($mg \cdot g^{-1}$)	Total P ($mg \cdot g^{-1}$)	Pigments ($SCDU \cdot g^{-1}$)	pl
1	Agnes	Polk	33.0	3.1	179	11.0	2.30	0.62	6.
2	Alligator	Osceola	32.1	2.4	164	11.6	1.96	0.08	6.
3	Apopka	Orange	64.1	3.5	325	28.6	1.46	0.82	8.
4	Aretta	Polk	22.3	2.6	113	10.5	1.63	0.50	6.
5	Ariana	Polk	31.1	3.5	156	14.0	1.53	0.82	8.
6	Baldwin	Orange	4.9	0.4	30	2.7	0.68	0.23	8.
7	Blue Cypress	Indian River	66.7	2.5	343	26.0	1.41	0.45	7.
8	Brooklyn	Clay	12.3	1.1	56	4.4	0.27	0.05	5.
9	Broward	Putnam	30.8	1.3	150	10.8	1.45	0.11	5.
10	Bryant	Marion	58.9	1.8	306	28.4	0.85	0.65	*
11	Buffum	Polk	30.2	2.2	173	8.8	1.98	0.16	5.
12	Butler	Orange	1.6	0.2	9	0.8	0.13	0.05	6.
13	Butler	Union	55.6	2.0	309	14.8	1.09	0.11	5.
14	Clay	Highlands	48.4	2.9	247	21.7	2.14	1.74	6.
15	Clinch	Polk	37.1	4.3	193	16.8	2.44	0.89	6.
16	Conway	Orange	35.1	4.0	179	15.0	3.67	0.60	7.
17	Cooter Pond 7	Alachua	59.2	2.2	309	26.8	4.56	2.37	6.
18	Cowpen	Putnam	34.8	2.2	175	13.0	1.44	0.25	5.
19	Crews	Pasco	59.8	2.7	318	29.2	0.92	1.52	6.
20	Crooked	Polk	37.9	3.1	210	15.4	1.70	0.27	6.
21	Crosby	Bradford	48.4	2.7	262	13.7	1.67	0.22	5.
22	Cypress	Osceola	31.5	2.9	158	18.8	1.82	3.77	6.
23	Damon	Highlands	25.3	2.2	128	7.2	1.14	0.18	6.
24	Deaton	Sumter	78.9	1.8	413	36.9	1.03	0.71	7.
25	Dinner	Highlands	8.1	0.6	45	4.0	0.40	0.13	8.
26	Doctors	Clay	35.8	4.5	178	17.8	1.34	0.66	8.
27	Down	Orange	3.2	0.4	20	1.3	0.19	0.03	6.
28	Eagle	Polk	41.0	2.2	210	18.9	1.95	1.99	7.
29	Eustis	Lake	30.6	0.3	183	16.6	0.59	0.18	7.
30	Fairview	Orange	8.2	0.9	38	3.4	1.55	0.12	8.
31	Galilee	Putnam	50.7	0.8	275	17.8	1.24	0.20	5.
32	Gentry	Osceola	42.6	1.7	237	12.8	1.15	0.28	5.
33	Gibson	Polk	35.9	2.0	203	11.4	3.75	0.39	7.
34	Glenada	Highlands	59.8	2.1	304	35.2	3.87	9.98	6.
35	Griffin	Lake	62.0	3.8	310	29.3	1.14	0.82	7.
36	Hampton	Bradford	63.6	2.3	345	21.1	1.37	1.51	5.
37	Harris	Lake	62.9	3.1	307	34.1	0.51	0.93	8.
38	Helen Blazes	Brevard	62.3	2.1	335	24.6	0.66	1.73	7.
39	Howard	Polk	40.7	1.7	222	21.4	1.84	1.12	8.
40	Huntley	Highlands	28.7	2.6	148	10.8	1.81	0.19	6.
41	Iola	Pasco	26.8	1.9	131	10.1	3.04	0.61	6.
41	Istokpoga	Highlands	3.8	0.5	21	1.7	0.18	0.03	6.
43	Jessamine	Orange	35.9	4.0	189	13.3	6.15	0.58	7.
44	Jessup	Seminole	40.7	3.7	200	21.2	2.05	1.43	8.
45	Johnson	Clay	47.8	3.1	267	19.0	1.14	0.27	5.
46	Josephine	Highlands	5.8	0.3	34	2.5	0.22	0.17	5.
47	June-In-Winter	Highlands	7.7	0.5	33	3.2	0.37	0.11	6.
48	Kissimmee	Osceola	0.8	0.1	4	0.6	0.07	0.03	7.
49	Lawne	Orange	35.0	3.4	172	12.6	3.67	0.06	7.

s of the variable. Water data are presented in Huber et al. (1982). Sediment data
ake sample. *Data unavailable.

	Water						
¹)	pH	Conduc-tivity (μS·cm⁻¹)	Alka-linity (mg·L⁻¹)	Secchi (m)	Chl *a* (mg·m⁻³)	Total N (mg·L⁻¹)	Total P (mg·L⁻¹)
	6.8	133	5.8	0.90	22.5	0.84	0.061
	6.1	110	4.0	1.78	3.4	0.63	0.023
	8.0	340	120.0	0.20	59.3	4.03	0.192
	6.8	199	7.5	2.90	4.8	0.49	0.020
	8.1	204	36.0	1.00	19.6	1.03	0.055
	8.6	206	97.3	1.50	17.4	0.57	0.034
	7.3	180	30.0	1.10	3.4	1.23	0.060
	5.5	32	1.5	3.43	2.1	0.19	0.011
	5.4	71	1.3	5.70	1.3	0.15	0.003
	*	74	*	*	*	0.80	0.030
	5.4	95	1.0	1.10	2.6	0.37	0.056
	6.0	207	2.0	6.00	1.3	0.44	0.012
	5.8	47	4.0	1.30	4.8	0.56	0.025
	6.8	235	12.5	0.89	5.1	0.71	0.027
	6.1	83	4.0	2.20	7.2	0.35	0.014
	7.3	193	41.0	3.70	4.2	0.62	0.020
	6.9	110	5.0	*	19.1	1.69	0.160
	5.2	61	0.0	3.48	1.6	0.19	0.010
	6.6	76	20.8	1.50	3.4	0.80	0.013
	6.4	90	2.9	2.60	3.6	0.43	0.016
	5.9	42	2.2	1.90	4.6	0.61	0.027
	6.6	135	10.0	0.60	81.0	1.62	0.090
	6.2	228	*	*	*	0.85	0.050
	7.4	165	24.7	0.50	34.4	2.82	0.032
	8.4	175	36.7	1.10	4.8	0.49	0.011
	8.2	2135	58.3	0.83	27.1	1.31	0.103
	6.5	208	7.0	6.20	1.0	0.69	0.017
	7.8	238	29.0	0.80	24.1	1.43	0.025
	7.8	272	103.0	0.40	32.3	2.40	0.064
	8.1	173	52.2	4.80	2.4	0.50	0.015
	5.6	50	1.0	2.96	1.6	0.34	0.018
	5.5	94	3.0	0.50	5.2	0.63	0.023
	7.4	105	16.0	0.80	15.0	1.03	0.204
	6.9	299	15.0	1.17	22.3	1.30	0.139
	7.8	261	102.0	0.40	51.2	2.55	0.100
	5.6	50	2.0	2.20	4.9	0.46	0.021
	8.4	225	88.3	0.80	19.0	1.35	0.030
	7.3	303	38.7	1.07	*	1.21	0.057
	8.7	164	59.0	0.30	122.6	2.05	0.048
	6.2	104	2.7	1.73	5.6	0.67	0.031
	6.8	130	16.0	2.90	5.3	0.50	0.022
	6.9	101	8.9	0.50	13.1	1.24	0.060
	7.7	184	43.5	3.40	5.7	0.62	0.012
	8.9	1059	78.0	0.52	104.0	3.07	0.420
	5.4	23	2.0	1.38	3.5	0.27	0.022
	5.8	83	5.0	0.80	10.8	0.86	0.060
	6.8	116	8.0	3.97	9.3	0.57	0.019
	7.1	118	19.0	1.60	20.7	1.36	0.047
	7.6	207	61.0	1.05	35.1	4.78	2.560

TABLE 1. (*Concluded*)

		Sediments						
Lake	County	LOI 550°C (%)	LOI 550–990°C (%)	Total C ($mg \cdot g^{-1}$)	Total N ($mg \cdot g^{-1}$)	Total P ($mg \cdot g^{-1}$)	Pigments ($SCDU \cdot g^{-1}$)	pH
50 Letta	Highlands	24.2	2.0	116	8.6	2.06	0.15	6.6
51 Lindsey	Hernando	84.2	1.4	463	36.9	1.12	1.39	*
52 Little Red Water	Highlands	39.8	2.9	208	11.7	1.68	0.49	6.0
53 Lochloosa	Alachua	56.6	2.4	282	31.9	1.68	1.42	7.2
54 Lotela	Highlands	15.1	1.8	73	5.7	1.02	0.12	7.1
55 Louise	Suwannee	83.5	0.3	476	27.9	1.28	2.05	6.4
56 Lowery	Clay	25.5	2.8	130	7.4	0.92	0.14	5.3
57 Lowery	Polk	76.3	3.6	442	33.4	0.87	1.00	6.2
58 Maitland	Orange	43.7	3.1	220	17.6	8.09	1.10	7.6
59 Mann	Orange	16.4	2.0	74	6.9	2.20	0.35	8.6
60 Margaret	Putnam	47.0	0.7	275	14.2	0.47	0.19	4.6
61 Marian	Osceola	55.2	1.9	315	28.6	1.79	0.76	7.0
62 Miccosukee	Jefferson	80.1	1.3	433	33.8	1.18	1.51	6.2
63 Minneola	Lake	1.8	0.2	14	0.9	0.19	0.02	6.6
64 Miona	Sumter	57.7	2.0	322	23.7	0.45	0.51	7.2
65 Moon	Pasco	52.0	2.9	280	22.1	0.65	0.92	6.6
66 Mystic	Madison	65.7	1.1	345	29.6	1.68	1.26	6.7
67 Ocean Pond	Baker	39.0	1.7	215	10.0	1.52	0.10	5.0
68 Okahumpka	Sumter	67.1	2.7	328	28.5	0.36	0.31	9.0
69 Orange	Alachua	70.1	2.3	352	35.4	1.23	1.18	7.2
70 Padgett	Pasco	11.9	1.8	50	5.0	0.69	0.29	6.8
71 Palestine	Union	70.4	0.6	409	18.6	1.00	0.16	4.9
72 Panasoffkee	Sumter	27.9	28.9	200	15.1	1.08	0.89	8.0
73 Pasadena	Pasco	41.7	1.1	224	16.7	1.06	0.17	6.4
74 Pierce	Polk	62.3	3.5	319	34.7	1.70	2.21	7.7
75 Red Beach	Highlands	40.6	2.0	222	11.3	0.62	0.24	6.6
76 Reedy	Polk	42.8	2.9	213	21.6	1.04	0.40	8.2
77 Rosalie	Polk	22.5	2.3	130	9.3	0.82	0.16	6.7
78 Sampson	Bradford	39.3	4.1	197	14.7	1.95	0.69	6.6
79 Santa Fe	Alachua	36.2	2.4	200	13.3	1.41	0.27	6.0
80 Sheelar	Clay	24.3	2.1	111	8.2	0.66	0.77	5.1
81 Smith	Marion	51.2	1.6	284	19.4	0.81	0.16	5.5
82 Stella	Putnam	14.0	1.0	71	5.6	0.51	0.09	7.1
83 Thonotosassa	Hillsborough	40.0	3.3	202	23.3	7.46	4.11	8.1
84 Tibet	Orange	2.9	0.2	18	1.4	0.19	0.03	6.1
85 Tiger	Polk	25.1	2.6	138	10.8	0.63	0.13	8.5
86 Tohopekaliga	Osceola	8.1	0.7	37	5.1	0.49	0.80	7.5
87 Townsend Pond	Lafayette	79.6	0.4	442	34.9	0.57	1.29	5.2
88 Tsala Apopka	Citrus	82.3	*	422	42.4	2.44	2.86	7.6
89 Underhill	Orange	27.3	4.0	128	11.9	8.05	1.39	7.5
90 Virginia	Orange	51.1	4.0	262	23.9	4.55	1.81	7.8
91 Wales	Polk	40.5	3.0	216	17.9	2.04	0.95	7.4
92 Washington	Brevard	40.9	1.9	209	15.7	0.48	0.12	*
93 Watertown	Columbia	60.3	1.9	340	22.5	2.13	2.92	7.4
94 Weohyakapka	Polk	5.3	0.5	29	2.2	0.29	0.03	7.0
95 Wildcat	Lake	49.1	2.9	263	18.1	1.25	0.90	4.8
96 Winnemisett	Volusia	16.2	1.1	81	6.2	0.74	0.15	6.7
97 Yale	Lake	52.7	2.3	263	27.2	0.62	0.68	8.2
Mean		39.7	2.4	210	16.7	1.60	0.83	
SD		21.9	3.0	119	10.3	1.54	1.24	
Minimum		0.8	0.1	4	0.6	0.07	0.02	4.6
Maximum		84.2	28.9	476	42.4	8.09	9.98	9.0

	Water						
	pH	Conductivity ($\mu S \cdot cm^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	Secchi (m)	Chl *a* ($mg \cdot m^{-3}$)	Total N ($mg \cdot L^{-1}$)	Total P ($mg \cdot L^{-1}$)
	6.6	125	5.1	1.28	3.7	0.40	0.040
	*	59	*	0.80	*	*	*
	6.0	50	3.2	0.70	16.3	0.47	0.032
	7.2	77	23.6	0.70	25.5	1.53	0.050
	7.1	26	13.1	2.20	3.1	0.75	0.019
	6.4	46	5.0	1.80	6.7	0.60	0.020
	5.3	23	1.5	3.31	1.5	0.18	0.013
	6.2	107	7.0	1.40	10.4	1.40	0.030
	7.6	205	55.1	1.80	6.4	0.67	0.040
	8.6	290	*	*	*	0.83	0.040
	4.6	59	0.4	0.71	5.5	0.48	0.019
	7.0	104	20.0	0.40	46.7	1.74	0.083
	6.2	30	5.2	*	6.0	0.94	0.042
	6.6	78	4.5	4.00	2.8	0.48	0.016
	7.2	138	24.7	1.10	12.1	1.37	0.018
	6.6	78	7.0	2.70	3.5	0.65	0.029
	6.7	31	10.0	*	3.6	0.51	0.015
	5.0	35	0.5	1.10	3.9	0.42	0.040
	9.0	177	51.3	1.20	5.1	0.95	0.020
	7.2	67	18.0	0.80	16.8	1.11	0.040
	6.8	134	16.0	3.60	2.4	0.57	0.017
	4.9	34	1.0	1.10	4.8	0.51	0.020
	8.0	231	110.5	1.26	5.5	0.61	0.035
	6.4	93	6.0	1.20	8.8	0.84	0.028
	7.7	120	36.1	0.30	21.3	1.13	0.031
	6.6	55	4.0	1.02	11.6	0.55	0.021
	8.2	174	48.0	1.40	28.2	1.55	0.046
	6.7	83	10.0	0.60	5.6	0.81	0.040
	6.6	124	9.7	2.00	6.4	0.67	0.043
	6.0	49	3.0	2.20	5.6	0.61	0.025
	5.1	19	*	7.94	1.3	0.19	0.005
	5.5	62	1.5	2.80	2.4	0.39	0.011
	7.1	239	16.0	4.10	2.0	0.43	0.010
	8.1	214	65.9	0.70	53.2	0.59	0.690
	6.1	197	3.0	*	3.2	0.44	0.013
	8.5	86	26.4	0.70	10.2	0.84	0.041
	7.5	171	25.0	0.40	71.0	1.69	0.322
	5.2	43	2.2	3.80	5.1	0.58	0.009
	7.6	109	56.6	2.90	2.8	1.10	0.070
	7.5	232	61.9	2.60	29.6	1.52	0.052
	7.8	245	67.2	8.20	21.3	0.64	0.028
	7.4	119	43.0	0.50	8.0	0.52	0.022
	*	428	*	0.80	*	*	*
	7.4	163	59.5	1.90	15.9	1.05	0.062
	7.0	76	11.0	1.00	5.2	0.65	0.040
	4.8	34	0.9	3.61	1.2	0.19	0.008
	6.7	173	7.0	5.80	1.0	0.48	0.010
	8.2	264	110.0	1.49	14.4	1.02	0.022
		165	25.8	1.96	15.2	0.94	0.079
		238	30.4	1.68	21.5	0.76	0.272
	4.6	19	0.0	0.20	1.0	0.15	0.003
	9.0	2135	120.0	8.20	122.6	4.78	2.560

TABLE 2. Correlation matrix relating surface sediment variables for 97 Florida lakes. Variables were expressed as amounts per gram of dry sediment. Table values are correlation coefficients (r). $^{**}P < 0.01$.

	C	N	P	SCDU
Organic matter	0.99**	0.93**	0.13	0.39**
C		0.91**	0.10	0.36**
N			0.16	0.52**
P				0.39**

TABLE 3. Correlation matrix relating sediment and water variables for 91 Florida lakes. Table values are correlation coefficients (r). $^{*}P < 0.05$; $^{**}P < 0.01$.

	Water variables		
Sediment variables	TSI-Chl *a*	TSI-N	TSI-P
% organic matter	0.20	0.27**	0.11
C·g dry^{-1}	0.18	0.25*	0.09
N·g dry^{-1}	0.35**	0.42**	0.20
N·g organic^{-1}	0.52**	0.49**	0.32**
P·g dry^{-1}	0.26*	0.13	0.36**
SCDU·g dry^{-1}	0.35**	0.25*	0.33**
SCDU·g organic^{-1}	0.45**	0.26*	0.42**

and watershed to lake ratio ($n = 67$). C:N ratio was inversely correlated with drainage area ($r = -0.39, P < 0.01, n = 68$). Midlake sediment C:N ratio also declines as a function of lake surface area ($r = -0.37$, $P < 0.01$, $n = 92$). The latter relationship suggests that with greater distance from the shoreline, sediments are increasingly dominated by low-C:N autochthonous organics as opposed to high-C:N allochthonous organics.

Total P in the sediments is uncorrelated with concentrations of total C, N, and organic matter (Table 2), suggesting that (1) P is present in both the organic and inorganic sediment fractions and/or (2) the P content of sediment organic matter is highly variable. We did not differentiate among the forms of sedimented P, but there is evidence that some lakes contain muds with significant amounts of inorganic P. Twenty-seven of the surveyed lakes have sediment N:P weight ratios <7, the value reported for plankton (Richards 1965; Wetzel 1975). Twenty-four of these lakes are in counties with phosphate-rich bedrock (Orange, Polk, Lake, Highlands) and probably receive inputs of P-rich allochthonous inorganics.

Pigments (SCDU's) are part of the organic sediment fraction. Nevertheless, their relatively weak correlation with organic matter content (Table 2) is further evidence of the high between-lake variability in organic matter pigment content. The variability may be a consequence of interbasin differences in the relative amounts of allochthonous and autochthonous organics as well as between-lake differences in pigment preservation.

Sediment – Trophic State Relationships

Concentrations of sediment constituents were related to trophic state of the overlying water using simple correlation (Table 3). Trophic state is expressed as Carlson's (1977) trophic state index (TSI) for Chl *a* and P. TSI-N is a N-based TSI index developed for Florida lakes (Kratzer and Brezonik 1981). Six lakes in the data set lacked Chl *a* measurements and were excluded from consideration.

Our data provide little support for the conclusion of Flannery et al. (1982) that Florida lakes of higher trophic state have greater proportions of organic matter in their surface sediments. Percent organic matter in the uppermost muds is uncorrelated with TSI-Chl *a* and TSI-P. The sediment variable is weakly correlated with TSI-N, explaining only 7% of the variance in the water variable (Table 3). Total C comprises a nearly constant proportion of the organic matter in Florida lakes, and therefore, correlations between total C and TSI measures yield almost identical r values as percent organic matter – TSI correlations.

TSI values reflect only water-column chlorophyll and nutrient concentrations. Sedimentary organic matter is derived from several autochthonous and allochthonous sources, some of which are poorly related to or wholly unrelated to midlake water column conditions. The weak correlation between organic content of the sediments and trophic state is thus unsurprising. Furthermore, diagenesis of organic sediments probably proceeds at different rates in various lakes of the survey. Nevertheless, it has been demonstrated that in Florida lakes, net accumulation rates of organic matter and total C are positively correlated with trophic state (Binford and Brenner 1986; Deevey et al. 1986). If allochthonous inorganic accumulation proceeds at similar rates in the surveyed lakes, positive correlations between organic content (or total C content) of the sediments and TSI should result. Lack of correlation between concentrations of these sediment constituents and TSI's indicates variable dilution of sedimentary organic matter by detrital inorganics.

Total N expressed per gram of dry sediment is uncorrelated with TSI-P and positively correlated with TSI-Chl *a* and TSI-N (Table 3). When N concentration is expressed as a proportion of the sediment organic matter, correlations with TSI measures are improved (Table 3). Interpretation of these correlations is confounded because N per gram of organic matter is also positively correlated with other water-column and morphometric variables: pH ($r = 0.57$, $P < 0.01$, $n = 94$), conductivity ($r = 0.28, P < 0.01, n = 95$), bicarbonate alkalinity ($r = 0.40$, $P < 0.01$, $n = 64$), and lake surface area ($r = 0.48, P < 0.01, n = 92$). Many of the limnological variables are in turn intercorrelated.

Flannery et al. (1982) found a strong negative correlation (-0.85) between sediment C:N ratio and ln Chl *a* in the water column. In our data set, the analogous C:N ratio – TSI Chl *a* correlation is somewhat weaker ($r = -0.43$, $P < 0.01$, $n = 91$). Interpretation of the relationship is confounded by the strong negative correlation between sediment C:N ratio and water-column pH ($r = -0.62, P < 0.01, n = 91$). When pH effects are removed by partial correlation, sediment C:N ratio and TSI-Chl *a* are uncorrelated.

Sedimentary P concentration is uncorrelated with TSI-N, weakly correlated with TSI-Chl *a*, and more strongly correlated with TSI-P (Table 3). Poor relationships with TSI-N and TSI-Chl *a* are not surprising. N:P ratios of the lake waters are highly variable among the lakes. Only 31 of the 91 lakes with reported TSI-Chl *a* values have N:P ratios >30, the criterion used by Huber et al. (1982) to establish P limitation of primary productivity.

Sedimentary P – TSI correlations can be weakened if washed-in large particulate P is significant in the sediment of some lakes but is unmeasured and unavailable to plankton in the overlying waters (Prepas and Vickery 1984). This scenario appears to be the case for those lakes located over phosphatic limestones. For a data set of 34 Florida lakes, net accumulation rate of P in sediments was positively correlated with TSI-P (Binford and Brenner 1986). Four outlier lakes having excessively high net

accumulation rates lie in the phosphate district and probably receive detrital particulate P that is removed quickly from the water column.

Pigment concentrations expressed per gram of dry sediment are positively correlated with both TSI-Chl *a* and TSI-P, but show a weaker relationship with TSI-N (Table 3). Pigments were also expressed per gram of organic matter to remove interlake variation in the dilution of organics by allochthonous inorganics. The procedure improves the correlation with TSI-Chl *a* and TSI-P, but leaves the relationship with TSI-N unchanged. Several processes may account for the poor correlations. The lakes probably differ with respect to the proportion of sediment organic matter derived from terrestrial, aquatic macrophyte, and phytoplankton sources. These sources differ in pigment content. Additionally, conditions for pigment preservation may differ among basins. Flannery et al. (1982) hypothesized better preservation to explain high pigment concentration per gram of organic matter in the sediments of two deep lakes. In our data set, the SCDU content of sediment organic matter was uncorrelated with the water depth from which samples were collected. While increasing depth may contribute to pigment preservation, some shallow lakes studied by Flannery et al. (1982), and other lakes in our survey, had high pigment concentrations in their organic deposits. In situ chlorophyll production by sedimented or epipelic algae may account for the high SCDU concentrations measured in sediments from shallow-water sites. Evidence comes from the fact that the proportion of native chlorophyll (the chlorophyll fraction undegraded to pheopigments: see Lorenzen 1967) is inversely correlated with water-column depth of the sediment sample ($r = -0.37, P < 0.01, n = 96$).

For Florida lakes, correlations between concentrations of sediment variables and trophic state are, at best, weak. Our best simple correlation relates the concentration of nitrogen per gram of organic matter to TSI-Chl *a*, but explains only 27% of the variance in the trophic state measure.

We used stepwise multiple regression to improve the ability of the sediment data to predict TSI measures. We selected the model that explained the greatest amount of variance in TSI, with the further requirement that all regression coefficients for independent variables be significant at the $P < 0.05$ level. Our best equation is as follows:

$$\begin{aligned}\text{TSI-Chl } a = \; & 31.71^{**} + 0.61^{**}\ \text{N}\cdot\text{g org}^{-1} \\ & + 4.85^{**}\ \text{P}\cdot\text{g dry}^{-1} \\ & - 1.36^{**}\ \text{P}\cdot\text{g org}^{-1} - 4.48^{*}\ \text{SCDU}\cdot\text{g dry}^{-1} \\ & + 2.29^{*}\ \text{SCDU}\cdot\text{g org}^{-1}\end{aligned}$$

where $^{*} = P < 0.05$, $^{**} = P < 0.01$, and nutrient concentrations are in milligrams per gram. The model explains 43% of the variance in TSI-Chl *a*. In Florida lakes, simple concentrations of sediment variables, whether alone or in combination, are poorly correlated with trophic state of the overlying waters. In related studies, we have demonstrated that net accumulation rates of elements in Florida lake sediments are more effective than simple concentrations as predictors of lake trophic state (Binford and Brenner 1986; Brenner and Binford 1986; Deevey et al. 1986).

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Measures of Optimal Thermal Habitat and Their Relationship to Yields for Four Commercial Fish Species

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Christie, G. C., and H. A. Regier. 1988. Measures of optimal thermal habitat and their relationship to yields for four commercial fish species. Can. J. Fish. Aquat. Sci. 45: 301–314.

Measures of thermal habitat space were developed by integrating, over time during the summer period, the amount of lake bottom area and pelagic volume with water temperatures within species' optimal thermal niches. These species' specific measures, thermal habitat area, THA (hectares per 10 d), and thermal habitat volume, THV (cubic hectometres per 10 d), were used as predictor variables in regression equations estimating the total sustained yield, SY (kilograms per year), of each of four commercially important species: lake trout, *Salvelinus namaycush*; lake whitefish, *Coregonus clupeaformis*; walleye, *Stizostedion vitreum vitreum*; and northern pike, *Esox lucius*. One or both of THA and THV were strongly correlated with SY for each of the four species for a set of 21 large north-temperate lakes. Several other habitat variables were assessed with respect to species' SY: total lake area and volume, mean depth, total dissolved solids, and the ratio of the latter two as R. A. Ryder's morphoedaphic index. The various statistical results are interpreted with respect to the ecology of the four species.

On a quantifié, au cours de la période estivale, l'espace thermique d'un habitat en intégrant la superficie du fond d'un lac et le volume d'eau pélagique à la température de l'eau des niches thermiques optimales. Ces quantifications particulières à chaque espèce, soit la superficie de l'habitat thermique (THA en hectares par 10 j) et le volume thermique de l'habitat (THV en hectomètres cubes par 10 j), ont servi de variables explicatives dans des équations de régression afin de déterminer le rendement soutenu total (SY en kilogrammes par année) de quatre espèces commercialement importantes : le touladi (*Salvelinus namaycush*), le grand corégone (*Coregonus clupeaformis*), le doré jaune (*Stizostedion vitreum vitreum*) et le grand brochet (*Esoc lucius*). Soit THA ou THV ou ces deux variables étaient en corrélation élevée avec le SY de chaque espèce dans une série de 21 grands lacs septentrionaux tempérés. On a aussi évalué plusieurs autres variables de l'habitat en fonction du SY de chaque espèce : superficie et volume totaux du lac, profondeur moyenne, concentration totale de solides dissous et rapport entre les deux dernières variables à titre d'incide morphoédaphique de R. A. Ryder. Les divers résultats statistiques sont interprétés en fonction de l'écologie des quatre espèces étudiées.

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The importance of temperature as a controlling variable at all levels of ecological systems is well known (Fry 1947; Precht et al. 1973). Yet, there are few generalizations about temperature effects at levels of organization above that of the organism. This work (see also Christie 1986) uses generalizations from species-specific thermal autecology in an investigation of the effects of environmental temperature on the production or yield of fish populations.

Our approach is to generate, from an understanding of thermal autecology, simple habitat or resource space measures which quantify temperature as a niche dimension (Magnuson et al. 1979; Kerr and Ryder 1977; Coutant 1987). To test the applicability of these measures, we use them as predictors of the productive capacity of large freshwater lakes, in terms of species-specific sustained yields.

Climate and environmental temperature are important variables determining the productivity of lakes. Rawson (1939) included the direct and indirect influences of these variables in a schema defining the trophic nature of lakes (see Steedman and Regier 1987). Rawson (1960, 1961) used mean temperature of the top 20 m to classify 12 large temperate lakes in a qualitative lake typology.

Ryder's (1965) morphoedaphic index (MEI), a ratio of the concentration of total dissolved solids divided by lake mean depth, provides first-order approximations of the aggregated productive capacities of freshwater systems. Ryder's original study examined total community fish yields within a relatively narrow range of climatic temperatures. Attempts to generalize MEI outside this range showed variation from the original relationship (Henderson et al. 1973; Ryder et al. 1974). This variation was partially explained for lakes over a wide range of latitude by the inclusion of long-term average annual air temperature as a predictor variable (Schlesinger and Regier 1982).

Environmental temperature has been entered into a number of other comparative studies with varying results. Community and species production in 294 reservoirs, distributed over the

southern United States, showed response to environmental temperature in terms of growing season as frost-free days (Jenkins 1968, 1982; Jenkins and Morais 1971). The responses of individual species yields and standing crops to the number of frost-free days were variable.

In his evaluation of models of lake productivity, Straskraba (1980) pointed to the important effect of latitude which determined temperature and light regimes. Over the wide range of latitudes in the lakes of the International Biological Programme (IBP), the variables affecting the availability of solar energy were found to be the most important in predicting lake primary productivity (Brylinsky and Mann 1973; Brylinsky 1980); significant relationships were found between primary productivity and latitude and mean annual air temperature.

Schlesinger and Regier (1983) found significant curvilinear responses of sustained yields of several individual species over a range of long-term mean annual air temperatures in the Matuszek–Ryder data set (Matuszek 1978). These humped distributions showed an optimum average annual air temperature for lake whitefish (−1.0°C), northern pike (1.5°C), and walleye (2.0°C). The relationship for lake trout did not show a significant curvilinear distribution, but there was evidence that the optimum air temperature was less than −1.0°C. These curves defined relatively narrow ranges of mean annual air temperatures in which lakes produced high yields of these species when compared with the ranges of temperatures over which the species were known to occur. These results, which are generally consistent with those of Magnuson and colleagues (see below), implied a route by which generalized population response to environmental temperature could be examined.

Temperature is a major "controlling factor" affecting the performance or activity of poikilothermic organisms (Fry 1947, 1971). The response of growth rate, expressed as the accumulation of biomass, to temperature has the same basic pattern as a generalized metabolic response (Brett 1979). Figure 1a presents the pattern of specific growth rate with temperature idealized after the work of Brett et al. (1969) on juvenile sockeye salmon (*Oncorhynchus nerka*). The maximum of this curve defines the optimum temperature for growth for this species. Figure 1b shows examples of growth with temperature data for three of the species examined here (see also Table 3).

While temperature exerts a controlling influence on fish, fish as responsive organisms exert control over their thermal experience. A species' behavioural thermal regulation is characterized by a single temperature to which it will ultimately gravitate with all other variables being equal or nonrestricting; Fry (1947) termed this the "final preferendum." Beitinger and Fitzpatrick (1979) and Magnuson et al. (1979) pointed to direct physiological correlates of this final preferendum, one of which is growth. This is borne out in the empiric assessments of the relation of optimal temperature for growth and final preferenda by McCauley and Casselman (1981) and Jobling (1981). Magnuson et al. (1979) defined a species' "fundamental thermal niche" as a range of temperature around the species' final preferendum which defines the optimum conditions for activity and metabolism, that is, a temperature range containing the maximum of the generalized growth/metabolic response curve (Fig. 1a). They quantified this fundamental thermal niche by examining the statistical distributions of temperatures selected by species in experimental evaluation of their thermal preferences. The average range of this niche for the species they examined was ±2° around the final preferendum.

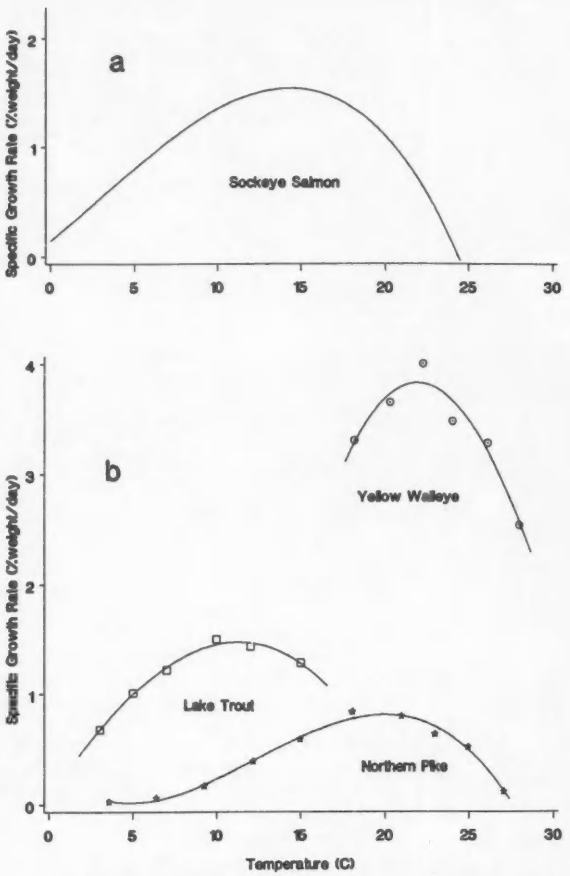

FIG. 1. (a) Relationship of growth rate and temperature for juvenile sockeye salmon, fed at ad libitum ration, after Brett et al. (1969). (b) Actual growth response to temperature at ad libitum ration for lake trout (O'Connor et al. 1981), walleye (Koenst and Smith 1976), and northern pike (Casselman 1978). Curves fitted to authors' original data. Table 3 gives the other growth temperature data found in the literature.

The distribution of temperature in temperate lakes is heterogeneous in time and space (Coutant 1987). We have attempted to quantify the thermal habitat available to a species in a given lake. We have developed measures of lake benthic area or volume with temperatures within the fundamental thermal niche of a species integrated over time. The seasonal lake thermal structure comes directly from temperature regime data and the space component is derived from the hypsographic (i.e. area versus depth) representation of lake basin morphometry.

To test the hypothesis that the pattern of environmental temperature in lakes affects the production and yield of individual species populations, we compare these time-integrated measures of optimal thermal habitat with reported long-term fish yields. Using data from 21 large north-temperate lakes, we have used these measures of thermal habitat as independent variables in regression analyses with estimates of the maximum sustainable yield of four commercial fish species: lake trout, *Salvelinus namaycush*; lake whitefish, *Coregonus clupeaformis*; walleye, *Stizostedion vitreum vitreum*; and northern pike, *Esox lucius*. The relationships between species' sustained yields and other

lake morphometric and edaphic variables are compared with these results. Finally, we assess the results of these statistical analyses with respect to the population ecology of the four species.

Materials and Methods

We used the set of large lakes and lake basins used by Schlesinger and Regier (1983) and by Matuszek (1978) (Table 1). These were the intensively fished lakes of Ryder's (1965) original set and include most of the large Saskatchewan lakes examined by Rawson (1960, 1961). They range in latitude from 42 to 62°N. Long-term commercial catch records and some records of temperature structure were available for these lakes.

Schlesinger and Regier (1983) pointed out that the relationships between the maximum catch statistics and true maximum sustained yield are imprecise and they propose the term sustained yield (SY) for the estimate of stable maximum catch for each species in a given lake. Like Schlesinger and Regier (1983), the estimates of SY we used are the maximum 15-yr running average annual catch by species, following Matuszek (1978) (Table 2). The intervals of years selected differ somewhat from those used by Schlesinger and Regier (1983) for reasons given in Christie (1986). The SY estimate for lake trout in Lake Erie's eastern basin is from Moenig et al. (1974). We have used the absolute average catch figures for SY here, that is, kilograms per year rather than the areal weighted version (kilograms per hectare per year) used by Schlesinger and Regier (1983) and most other studies. Using this absolute estimate for SY allows the explicit examination of the effect of lake size on this absolute catch (Youngs and Heimbuch 1982).

For each species, we selected a 4° optimal temperature range, approximating the fundamental thermal niche of Magnuson et al. (1979). Many authors have observed that the temperatures selected by fish in the field are consistently less than laboratory-determined final preferenda (e.g. Ferguson 1958; Rudstam and Magnuson 1985). Factors contributing to this difference include acclimation effects, seasonal patterns in behavioural thermoregulation, lower ration levels available in the field compared with the ad libitum levels used in most laboratory studies, and the use of young fish in most laboratory studies (see Christie 1986). To accommodate for these influences, we selected a 4° range skewed down approximately 1° from the symmetric construct around the optimum temperature for growth and the final preferendum suggested by Magnuson et al. (1979). Table 3 lists the laboratory-determined values of the final preferendum and the optimum temperature for growth from studies of the thermal autecology of each of the four species. The averages of the values of both measures are presented, along with the 4° optimal temperature range that we selected for each species.

The bottom area and the pelagic volume in a lake within each species' thermal niche were calculated from isotherm representations of the seasonal thermal structure and the hypsographic, cumulative area versus depth, curve for the lake. The amount of data available on water temperature profiles varied among the lakes in the data set (Table 4). To enable comparison between the lakes the measures were developed using only data from the summer period (i.e. June 5 – September 2), during which water temperature surveys were most consistently available (Christie 1986). While habitat with temperatures within the species' optimal temperature ranges occurred after the September 2 cutoff, especially for the cold-water species lake trout and lake whitefish, Christie (1986) found no reason to conclude that this cut-off should influence this comparative investigation. The development of lake water temperature models to describe the full seasonal temperature cycle would allow inclusion of the full occurrence of any given temperature range in time and would therefore produce more complete measures of optimal habitat available.

For a given lake in a given season, water temperature values available were averaged by depth at 10-d intervals. There were no areal or volumetric weightings to account for sampling dis-

TABLE 1. Descriptive data for lakes including the morphoedaphic index (MEI) (Ryder 1965), long-term mean annual air temperature (TEMP), and identifier (lake code). Data from Matuszek (1978) and Schlesinger and Regier (1983).

Lake	Lake code	Area ×1000 (ha)	Volume ×1000 (hm^3)	Maximum depth (m)	Mean depth (m)	TDS (mg/L)	MEI ($mg \cdot L^{-1} \cdot m^{-1}$)	TEMP (°C)
Great Slave Lake	A	2538.2	1574.0	614.0	62.0	135.0	2.2	−4.4
Amisk Lake	B	32.1	4.2	40.0	13.2	88.0	6.7	−0.4
Wollaston Lake	D	206.2	42.5	97.0	20.6	35.0	1.7	−3.3
Reindeer Lake	E	556.9	94.7	215.0	17.0	39.0	2.3	−5.2
Big Peter Pond	F	55.2	7.6	24.0	13.7	138.0	10.1	−5.2
Little Peter Pond	G	18.9	1.0	9.5	5.1	144.0	28.2	−0.6
Churchill Lake	H	43.3	3.9	24.0	9.0	136.0	15.1	−0.6
Cree Lake	I	115.2	17.2	60.0	14.9	27.0	1.8	−2.3
Lake la Ronge	J	130.7	17.7	42.0	12.7	149.0	11.7	−0.8
Lac Île-à-la-Crosse	K	44.6	3.7	32.5	8.2	185.0	22.6	−0.6
Rainy Lake	L	85.8	8.5	49.1	9.9	57.0	5.8	2.2
Lake of the Woods	M	356.9	28.2	65.8	7.9	92.0	11.7	3.3
Lake Superior	N	8241.4	12230	358.4	148.0	60.0	0.4	3.9
Lake Huron	O	4165.9	2791.0	217.6	61.0	108.0	1.8	6.6
Georgian Bay	P	1375.2	660.0	164.6	51.0	108.0	2.1	6.2
North Channel	Q	455.0	88.0	76.8	22.0	85.0	3.9	5.4
Lake Erie	R	2569.0	483.0	64.0	18.5	152.0	8.2	9.4
Lake Ontario	S	1947.7	1637.0	235.9	80.5	148.0	1.8	8.1
Lake Michigan	T	5801.6	4908.0	281.6	85.0	134.0	1.6	7.7
Lake Nipigon	U	448.1	221.5	127.0	49.4	98.0	2.0	0.0
Red Lakes	V	116.9	6.4	10.7	5.5	190.5	34.7	3.6

TABLE 2. Commercial yield data: the maximum 15-yr running average annual catch of each species. The ap
are presented.

Lake	Lake trout			Lake whitefish			
	Years	Catch ×1000 (kg/yr)	Data source(s)[a]	Years	Catch ×1000 (kg/yr)	Data source(s)[a]	
Great Slave Lake	1946–60	1003.2	1, 2	1948–62	1885.1	1, 2	1
Amisk Lake	1930–44	13.4	3	1931–45	97.2	3	1
Wollaston Lake	1957–71	159.2	4, 5	1959–73	125.7	4, 5	1
Reindeer Lake	1955–69	278.6	6	1957–71	456.8	6	1
Big Peter Pond		0.0	4, 7, 8	1950–64	336.7	4, 7, 8	1
Little Peter Pond		0.0	4, 7, 8	1941–55	69.2	4, 7, 8	1
Churchill Lake		0.0	4, 7, 8	1926–41	138.7	7, 8	1
Cree Lake	1945–59	100.6	4, 5				
Lake la Ronge	1925–39	64.0	9, 10	1957–71	193.3	4	1
Lac Île-à-la-Crosse		0.0	4, 11	1965–69	57.6	4, 11	1
Rainy Lake		0.0	13, 14	1924–38	56.8	13, 14	1
Lake of the Woods	1924–38	7.8	15, 16	1929–43	137.7	15, 16	1
Lake Superior	1919–33	1895.5	19, 20	1885–99	1097.3	19, 20	1
Lake Huron	1923–37	1499.7	19, 21	1923–37	1041.5	19, 21	1
Georgian Bay	1924–38	660.1	19, 21	1925–39	550.1	19, 21	1
North Channel	1925–39	254.8	19, 21	1924–38	104.7	19, 21	1
Lake Erie	<1880	50.0	22	1939–53	1776.5	19, 23, 24	1
Lake Ontario	1914–28	311.6	19, 25	1915–29	798.6	19, 25	1
Lake Michigan	1911–25	3248.9	19, 26	1921–35	1218.3	19, 26	1
Lake Nipigon	1918–32	130.9	15	1918–32	501.7	15	1
Red Lakes		0.0	18				1

[a]Data sources: 1, records supplied to J. Matuszek by R. W. Moshenko, Freshwater Institute; 2, Keleher
J. Matuszek by F. M. Atton, Saskatchewan Department of Tourism and Renewable Resources; 5, Rawso
Kallemeyn 1973; 9, Rawson 1961; 10, Chen 1979; 11, Saylor 1972; 12, Chen 1980; 13, records supplie
Ministry of Natural Resources; 14, Chevalier 1977; 15, records compiled by J. Ridgeley, Ontario Minist
Smith 1972; 17, Schupp and Macins 1977; 18, Smith 1977; 19, Baldwin and Saalfeld 1970; 20, Lawrie an
22, Moenig et al. 1974; 23, Hartman 1973; 24, Leach and Nepszy 1976; 25, Christie 1973; 26, Wells and

. The applicable years and the sources of the data

a (s)[a]	Walleye			Northern pike		
	Years	Catch ×1000 (kg/yr)	Data source(s)[a]	Years	Catch ×1000 (kg/yr)	Data source(s)[a]
	1961–75	10.9	1, 2	1960–74	132.2	1, 2
	1931–45	4.2	3	1931–45	5.4	3
	1957–71	4.3	4, 5	1960–74	6.5	4, 5
	1956–70	20.3	6	1959–73	21.1	6
8	1941–55	85.5	4, 7, 8	1941–55	60.8	4, 7, 8
8	1957–71	57.1	4, 7, 8	1941–55	34.0	4, 7, 8
	1956–70	28.3	7, 8	1941–55	51.5	4, 7, 8
		.			.	
	1960–74	15.2	4, 12	1922–36	84.	4, 9
	1957–71	39.1	4, 11	1960–74	54.8	4, 11
4	1924–38	120.3	13, 14	1926–40	95.0	13, 14
5	1930–44	596.3	15, 16, 17	1925–39	445.0	15, 16
0	1953–67	100.0	19	1914–28	21.7	19
1	1930–44	776.8	19, 21	1923–37	14.9	19, 21
1	1923–37	43.6	19, 21	1923–37	41.3	19, 21
1	1924–38	49.5	19, 21	1924–38	36.4	19, 21
3, 24	1944–58	3612.8	19, 23, 24	1900–14	749.2	19, 23, 24
5	1951–65	49.4	19, 25	1900–14	132.0	19, 25
5	1945–59	241.9	19, 26	1903–30	35.5	19, 26
	1950–64	82.5	15	1951–65	10.4	15
	1938–52	353.2	18			

Keleher 1972; 3, Dean 1975a; 4, records supplied to
Rawson 1959; 6, Dean 1975b; 7, Rawson 1957; 8,
supplied to J. Matuszek by J. R. Chevalier, Ontario
Ministry of Natural Resources; 16, Heyerdahl and
wrie and Rahrer 1972; 21, Berst and Spangler 1973;
ells and McLain 1972.

TABLE 3. Values of the final preferendum and optimal temperature for growth for the four species from the literature. The mean values and the 4° optimal temperature ranges chosen are presented.

Species	Final preferendum (°C)	Source[a]	Optimal temperature for growth (°C)	Source[a]	Optimal temperature range (°C)
Lake trout	10.8	1	10–12	5	
	11.5	2	16	6	
	11.7	3			
	12.7	4			
Mean	11.7		12.7		8–12
Lake whitefish	12.7	7	12	10	
	13.5	8	15.1	11	
	14.3	9			
Mean	13.4		13.0		10–14
Walleye	23	11	20	12	
			22	13	
			22	14	
			22	15	
Mean	23		21.5		18–22
Northern pike	23–24	16	19	17	
			25	18	
			26	19	
Mean	23.5		23.3		19–23

[a]Data sources: 1, Peterson et al. 1979; 2, Goddard et al. 1974; 3, McCauley and Tait 1970; 4, Mac 1985; 5, O'Connor et al. 1981; 6, Gibson and Fry 1954; 7, Tompkins and Fraser 1950; 8, Opuszynski 1974, cited in Spotila et al. 1979; 9, Hoagman 1974; 10, Bernatchez and Dodson 1985; 11, Jobling 1981; 12, Kelso 1972; 13, Huh et al. 1976; 14, Koenst and Smith 1976; 15, Nickum 1978; 16, McCauley and Casselman 1981; 17, Casselman 1978; 18, Bevelhimer et al. 1985; 19, Hokanson et al. 1973.

tribution applied in this averaging. Linear interpolation was used to fill in depths or time intervals not represented by temperature measurements. Figure 2a is an example of the isotherm representation of a seasonal temperature regime averaged by this method. The isotherm diagrams for all lakes and years used are presented in Christie (1986). The hypsographic curves for each lake (Fig. 3) were derived from the literature, measured, or estimated (Christie 1986).

For each 10-d interval over the summer period the upper and lower depths corresponding to each species' thermal niche were measured (Fig. 2b). The area of lake bottom and the pelagic volume within these depth ranges were measured from the hypsographic curves. The integrated areas and volumes available over the summer period were approximated by summing all the interval values. We have called these time-integrated measures, of area and volume within the species' fundamental thermal niche, thermal habitat area (THA) and thermal habitat volume (THV), respectively. The units of these measures are hectares per 10 d and cubic hectometres per 10 d, respectively. The calculated THA and THV values were averaged over the years of available data for each lake to provide comparable measures of each lake's characteristic thermal structure (Tables 5 and 6).

The thermal habitat measures were used as predictor variables in regression analyses against the dependent variable total SY (kilograms per year) for each of the four commercial species. Other measures of lake size and shape were also considered as independent variables contributing to the species' SY (Table 1). For a discussion of discrepancies in values for area and the other variables reported in the literature, see Christie (1986).

While other, and perhaps better, edaphic or fertility measures were available for some of the lakes, only the concentrations of total dissolved solids (TDS) were available for the complete lake set. None of the lakes contained large concentrations of sulphates, chlorides, or other ions of low biological activity. The trend in time in TDS values is considered inconsequential in all but the Great Lakes for which it is compensated using the trend analysis of Beeton (1965) to the period for which the SY values are drawn (following Matuszek 1978).

Macrobenthos biomass was found to be the strongest single predictor of community yields (kilograms per hectare per year) in approximately the same data set by Matuszek (1978) and Hanson and Leggett (1982). The latter authors included observations for 16 of the lakes used in this study. Preliminary examination of this variable showed that it was correlated to some extent with the various THA and THV values, implying a temperature modulation of benthic production. This correlation would confound the effect of this benthos variable as a predictor of species' SY with the measures of thermal habitat and lake fertility. TDS was not correlated with the thermal habitat measures. For similar reasons the MEI was not included in multiple regression analyses with the thermal habitat measures. It was not independent of THA and THV (i.e. it was positively correlated with these measures), presumably because of the inclusion of a lake depth component in both measures.

All variables were found to be normalized with $\log_{10}$ transformations and were thus transformed before entry into the analyses. Unless noted, all reference to variables is to their $\log_{10}$ transformations. Probabilities reported for correlation or regression are *F*-test probabilities, unless specified otherwise.

TABLE 4. Specifics of water temperature data available for the lakes: the years for which profiles were available with the data sources by year, the number of years, the average number of stations sampled during the season (April–October), the coverage of this sampling regime (surface area per station), and the average number of dates sampled per station over the season. The Lake Michigan temperature data were averaged by the source authors; the values presented are approximations based on the averaging technique used.

Lake	Years (source(s)[a])	Number of years	Stations per season	Station coverage (× 1000 ha) per station	Dates sampled per station per season
Great Slave Lake	1944–48 (1), 1949–54 (2)	11	6	423.0	4
Amisk Lake	1950–51 (3), 1972 (4)	3	3	10.7	11
Wollaston Lake	1956 (5), 1972 (6)	2	9	22.9	7
Reindeer Lake	1954 (7), 1970–72 (8)	4	3	185.6	5
Big Peter Pond	1952–53 (9), 1969–70, 1972 (10, 11)	5	1	55.2	7
Little Peter Pond	1952–53 (9), 1969–70, 1972 (10, 11)	5	1	18.9	8
Churchill Lake	1952–53 (9), 1969–70 (11)	4	1	43.3	9
Cree Lake	1955 (5, 12)	1	6	19.2	5
Lake la Ronge	1948–52 (13, 14, 15)	5	3	43.6	5
Lac Île-à-la-Crosse	1954 (9), 1972 (16)	2	6	7.4	7
Rainy Lake	1969–72 (17)	4	4	21.5	10
Lake of the Woods	1962–65 (18, 19, 20, 21), 1980 (22)	5	4	89.2	10
Lake Superior	1971, 1973, 1983 (30)	3	128	64.4	4
Lake Huron	1970–71, 1974, 1980 (30)	4	94	44.3	3
Georgian Bay	1970–71, 1974, 1980 (30)	4	55	25.0	4
North Channel	1970, 1974, 1980 (30)	3	15	30.3	5
Lake Erie	1968–70, 1972, 1975, 1977–80, 1983 (30)	10	102	25.2	5
Lake Ontario	1966–70, 1972, 1974–83 (30)	15	96	20.3	7
Lake Michigan	1964–67 (31, 32), 1976 (33)	5	100	58.0	4
Lake Nipigon	1921–24 (23, 24, 25, 26), 1980–81 (27, 28)	6	5	89.6	3
Red Lakes	1955–57 (29)	3	1	116.9	23

[a]Data sources: 1, Rawson 1950; 2, Rawson 1956; 3, Rawson 1952; 4, Dean 1975a; 5, Rawson 1959; 6, Sawchyn 1973; 7, Novakowski 1955; 8, Dean 1975b; 9, Rawson 1957; 10, Kallemeyn 1972; 11, Kallemeyn 1973; 12, Rawson 1958; 13, Rawson and Atton 1951; 14, Atton 1955; 15, Rawson and Atton 1953; 16, Saylor 1972; 17, records supplied by B. Darby and D. McCleod, Ont. Minist. Nat. Res., Fort Francis; 18, Ont. Dep. Lands and Forests 1962; 19, Ont. Dep. Lands and Forests 1963; 20, Ont. Dep. Lands and Forests 1964; 21, Chambers and Macins 1965; 22, Foote et al. 1981; 23, Clemens 1923; 24, Clemens 1924a; 25, Clemens 1924b; 26, Dymond 1926; 27, Borecky 1981; 28, Borecky et al. 1982; 29, records supplied by G. R. Spangler, Univ. of Minnesota, Minneapolis; 30, records supplied by F. M. Boyce and F. C. Elder, Can. Centre for Inland Waters, Burlington; 31, Noble 1967; 32, Noble and Ewing 1968; 33, Bartone and Schelske 1982.

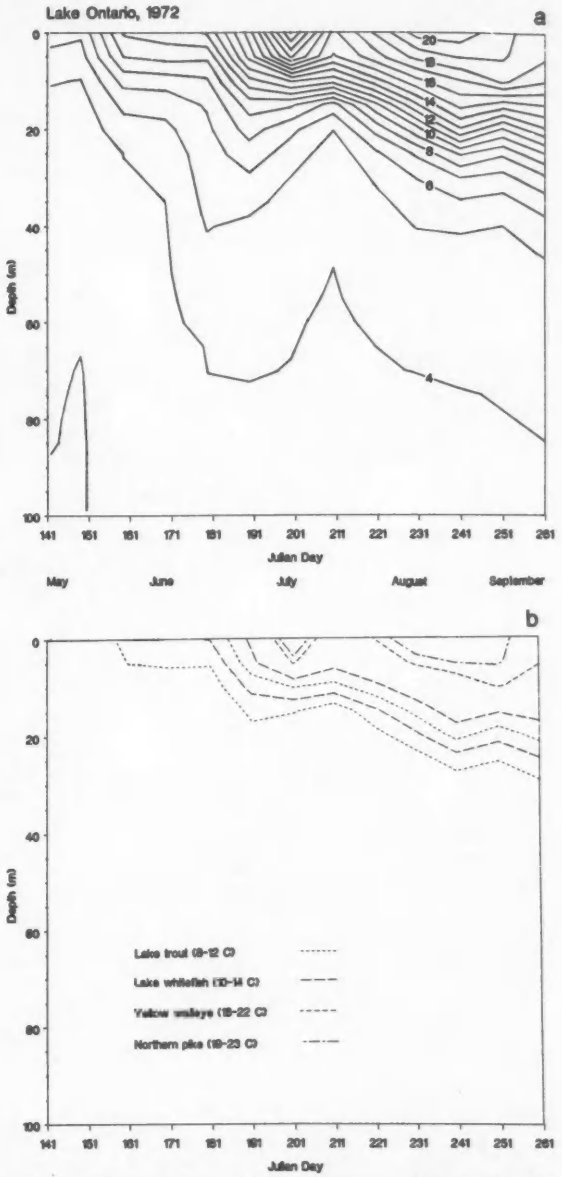

FIG. 2. (a) Example seasonal isotherm diagram, Lake Ontario, 1972. The water temperature has been averaged by depth over the whole lake at 10-d intervals and plotted at the midpoint of each interval (see text for description). (b) Specific optimal temperature ranges for the four species taken from the isotherm diagram above. The thermal habitat measures were derived from the depths associated with these temperatures at 10-d intervals from Julian day 156 to 245 (June 5 –September 2). Note that the lake surface forms the upper bound of the northern pike and walleye temperature ranges.

The correlation and regression analyses were all carried out with SAS procedures on the University of Toronto IBM computing facilities

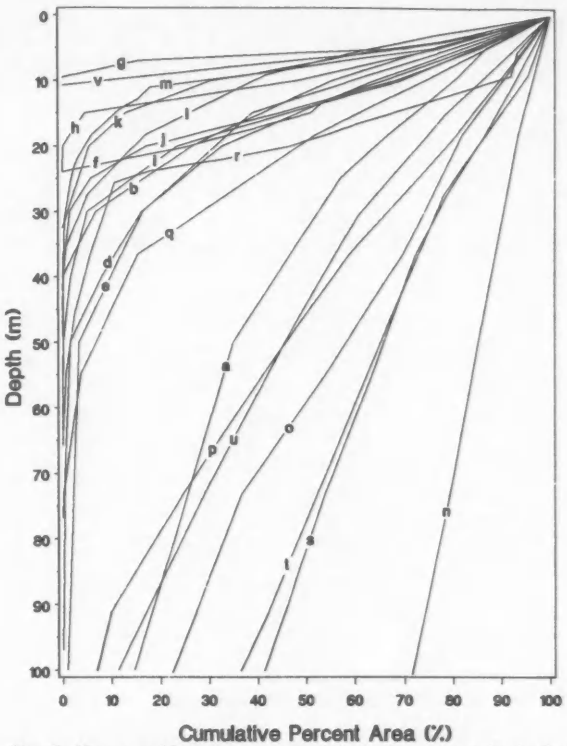

FIG. 3. Hypsographic curves, percent lake surface area at depth, for all 21 study lakes. Curves presented to 100 m. For maximum depth, lake surface area, and identifying code values, see Table 1. For the complete hypsographic data used, see Christie (1986). Data sources: a, Rawson 1950; b, Dean 1975a; d, Rawson 1959; e, Dean 1975b; f,g, Rawson 1957, extended to 5-m intervals with planimetry; h, Kallemeyn 1973; i, Rawson 1959; j, Rawson and Atton 1951; k, Saylor 1972; l, records supplied by B. Darby and D. McLeod, Ont. Minist. Nat. Resour., Fort Frances; m, records supplied by V. Macins, Ont. Minist. Nat. Resour., Fort Frances, and Carlander 1942; n,o,p,q,r,s,t, Rodgers 1964 and Anderson 1961; u, planimetry and approximation using the Neuman (1959) technique; v, approximation using the Hakanson (1977) technique.

Results

For all four species, SY was significantly correlated with THA ($p << 0.01$). Figure 4 presents the plots of these relationships. The coefficients of determination (r^2) show lake trout to have the strongest relationship followed by walleye, whitefish, and pike. Figure 5 presents SY versus THV. The relationship for pike is not significant (F-test, $p > 0.05$), while all the other regressions are significant ($p << 0.01$). THA is a better predictor of SY than is THV for walleye and pike, and THV is slightly better than is THA for lake trout and lake whitefish.

It would be expected that lake size, or maximum available habitat, would have a bearing on total catch or yield (SY, kilograms per year). This simple premise is generally accepted in the literature and accounted for by using yield per unit area (kilograms per hectare per year) as the dependent variable in production models (e.g. Ryder 1965; Matuszek 1978); the premise has been criticized by Youngs and Heimbuch (1982). Lake size as area or volume is a strong predictor of lake trout

TABLE 5. Thermal habitat area (THA): the benthic area available within the species specific optimal temperature range for growth, measured at the midpoint of 10-d intervals, summed over the summer period (June 5–September 2 (Julian days 156–245)), and averaged for the years of available data. The units of THA and its standard error (SE) are hectares per 10 d (summer), presented here in thousands.

	Lake trout		Lake whitefish		Walleye		Northern pike	
Lake	THA	SE	THA	SE	THA	SE	THA	SE
Great Slave Lake	2552.4	369.5	1769.4	247.7	91.6	72.3	45.7	37.5
Amisk Lake	91.4	10.3	89.8	10.5	11.8	5.5	6.3	3.8
Wollaston Lake	494.8	188.6	564.0	6.6	0.0	—	0.0	—
Reindeer Lake	952.0	88.8	1171.7	144.8	34.2	34.2	0.0	—
Big Peter Pond	127.0	12.5	137.8	16.0	25.3	17.1	9.2	4.8
Little Peter Pond	1.9	1.9	9.7	4.3	61.9	16.0	26.0	8.7
Churchill Lake	34.5	10.1	63.9	9.9	78.2	44.3	48.4	25.8
Cree Lake	240.5	—	236.5	—	0.0	—	0.0	—
Lake la Ronge	380.7	16.3	380.5	31.1	51.1	19.4	7.1	4.4
Lac Île-à-la-Crosse	32.3	19.1	40.5	22.9	81.8	60.3	27.5	25.7
Rainy Lake	136.2	25.6	128.0	22.7	300.7	25.4	262.8	30.7
Lake of the Woods	313.0	126.3	427.8	90.8	1506.9	276.3	1148.0	32.1
Lake Superior	2158.4	194.5	1134.0	310.4	122.8	123.1	93.1	93.1
Lake Huron	2649.5	199.7	2199.2	78.3	1203.1	62.5	464.5	197.7
Georgian Bay	1266.6	127.1	975.4	57.0	67.2	34.1	0.0	—
North Channel	1006.7	76.3	865.7	37.4	108.8	76.9	0.0	—
Lake Erie	743.2	61.4	6333.4	353.1	3757.7	354.3	3431.4	270.1
Lake Ontario	1006.6	37.2	769.7	37.2	481.5	56.5	334.0	56.8
Lake Michigan	4011.8	209.9	2812.6	142.1	778.0	340.7	377.1	196.6
Lake Nipigon	387.2	69.8	393.6	74.6	25.3	11.2	10.1	6.5
Red Lakes	16.8	16.8	29.6	20.1	532.8	16.4	632.0	82.5

TABLE 6. Thermal habitat volume (THV): the pelagic volume available within the species specific optimal temperature range for growth, measured at the midpoint of 10-d intervals, summed over the summer period (June 5 – September 2 (Julian days 156–245)), and averaged for the years of available data. The units of THV and its standard error (SE) are cubic hectometres per 10 d (summer), presented here in thousands.

	Lake trout		Lake whitefish		Walleye		Northern pike	
Lake	THV	SE	THV	SE	THV	SE	THV	SE
Great Slave Lake	1192.3	177.4	814.0	127.3	37.4	32.2	22.7	20.9
Amisk Lake	10.1	109.0	12.1	2.1	2.2	1.0	1.1	0.7
Wollaston Lake	88.7	35.3	110.9	4.9	0.0	—	0.0	—
Reindeer Lake	187.8	31.0	210.2	37.1	7.4	7.0	0.0	—
Big Peter Pond	10.0	12.9	13.3	1.1	5.3	3.3	2.3	1.1
Little Peter Pond	0.0	—	0.2	0.1	2.4	0.6	1.5	0.5
Churchill Lake	2.0	0.6	4.7	0.7	6.6	3.3	4.7	2.3
Cree Lake	28.9	—	34.2	—	0.0	—	0.0	—
Lake la Ronge	39.9	2.5	42.1	0.7	14.6	3.3	2.3	2.3
Lac Île-à-la-Crosse	1.9	1.2	3.7	2.4	9.0	5.7	3.3	3.0
Rainy Lake	13.2	3.1	12.9	2.4	34.1	2.9	29.2	3.5
Lake of the Woods	18.2	7.4	36.6	9.5	203.4	79.3	159.2	75.5
Lake Superior	6111.7	583.8	3138.9	881.7	331.9	331.9	252.3	252.2
Lake Huron	2600.3	207.5	2095.0	84.0	1165.8	62.9	452.9	193.4
Georgian Bay	937.0	87.7	769.9	43.9	60.5	30.2	0.0	—
North Channel	240.1	19.6	232.2	11.9	38.4	27.2	0.0	—
Lake Erie	203.8	17.2	714.7	57.3	1305.8	96.9	1465.3	109.7
Lake Ontario	1152.2	39.5	915.5	42.8	633.0	73.0	441.0	74.0
Lake Michigan	4406.8	260.0	3240.9	159.5	1251.5	476.3	657.6	321.2
Lake Nipigon	242.9	42.2	245.8	46.6	17.0	7.5	6.7	4.4
Red Lakes	0.1	0.1	0.4	0.4	23.1	6.9	28.2	4.5

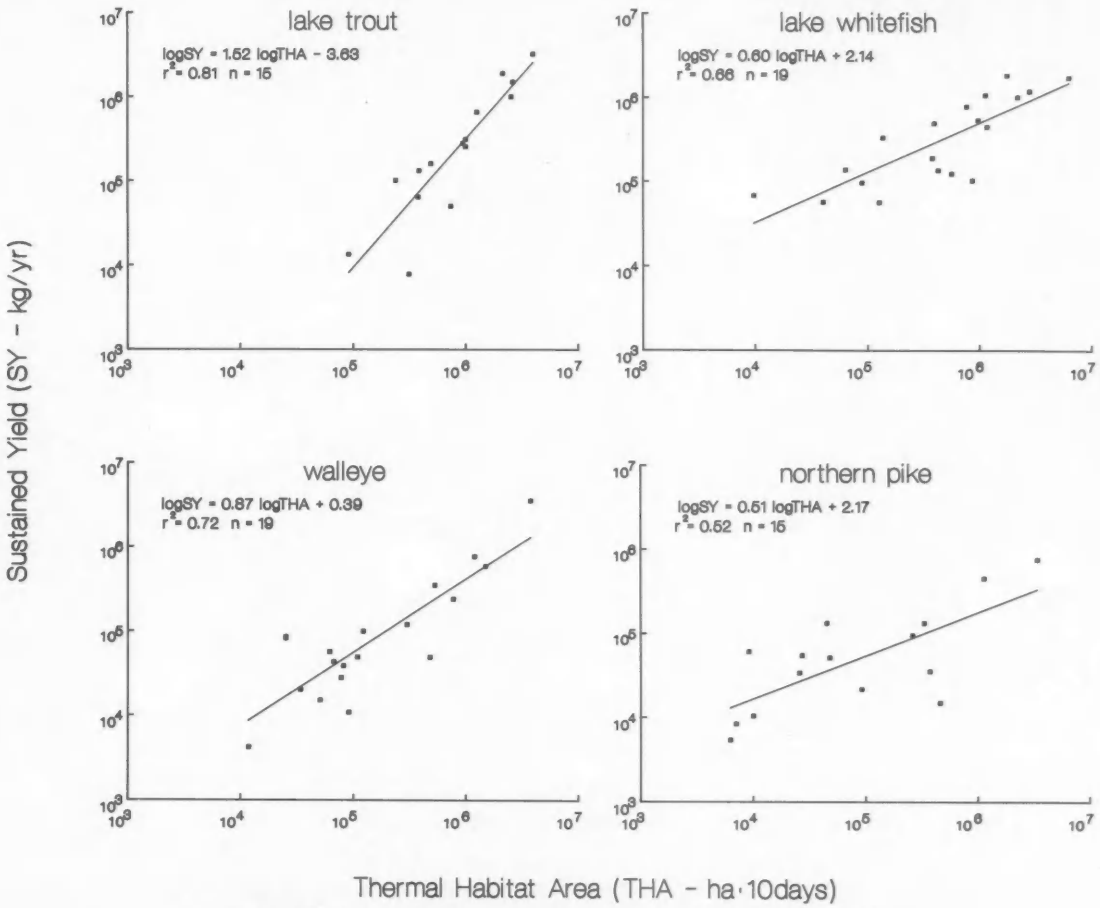
lake trout
logSY = 1.52 logTHA - 3.63
r² = 0.81 n = 15
lake whitefish
logSY = 0.60 logTHA + 2.14
r² = 0.66 n = 19
walleye
logSY = 0.87 logTHA + 0.39
r² = 0.72 n = 19
northern pike
logSY = 0.61 logTHA + 2.17
r² = 0.52 n = 15
Sustained Yield (SY - kg/yr)
Thermal Habitat Area (THA - ha·10days)

FIG. 4. Plots of total sustained yield versus thermal habitat area for the four species.

and whitefish total SY (see Table 7). The two warmwater species, walleye and pike, do not show significant regressions between either area or volume and SY ($p >> 0.05$) (see Table 7).

Pike and walleye show significantly stronger correlations between SY and THA than between SY and area or volume (Fisher's z-transformation, $p < 0.05$) (see Table 8). THA is weakly correlated with area and volume for both species. These two points lead to the conclusion that THA contributes significantly more information to the prediction of SY than lake size alone. These differences are not clear for lake trout and lake whitefish; the differences in correlation coefficients (r) for SY versus THA or THV or SY versus area and volume for lake trout and whitefish are not significant (Fisher's z-transformation, $p > 0.05$) (see Table 8). It is not clear how strongly the thermal habitat constructs developed here influence the SY of these cold-water species in these lakes.

The measures of optimal thermal habitat developed here are correlated with the total area and volumes of the lakes in this set (Christie 1986). Thus, to define more clearly the relative influence of the availability of water of the species' optimal thermal niche on SY, we converted the THA and THV values to unitless proportions of the total lake area and volume. The THA and THV values were divided by the total lake area and volumes, respectively, summed over the nine intervals of the summer period. The resulting indices (THAI and THVI) are time-integrated proportions of the total lake area or volume with temperatures within the species' thermal niche. By removing the direct area or volume effect from these indices, they can be considered independent of area and can be entered in multiple regression analyses with area.

Neither of these indices significantly predict SY when used alone in simple equations ($p > 0.05$) for any of the species (Christie 1986). The r^2 values for the multiple regression equations for SY with area, volume, and the thermal habitat indices, THAI and THVI, are presented in Table 8 (see Christie (1986) for full equations). The addition of THAI to area or THVI to volume in the equations for lake trout SY substantially improves the amount of the variation accounted for by simple area or volume (Table 8), i.e. increases the information content by 10.5 and 15.0%, respectively. For whitefish, there is virtually no increase in information with the addition of these indices to the simple regressions of area and volume. For walleye and pike, the greatly increased explanatory power was expected given the strength of the THA and THV versus SY relationships. Area is the most important lake size measure for both species. The

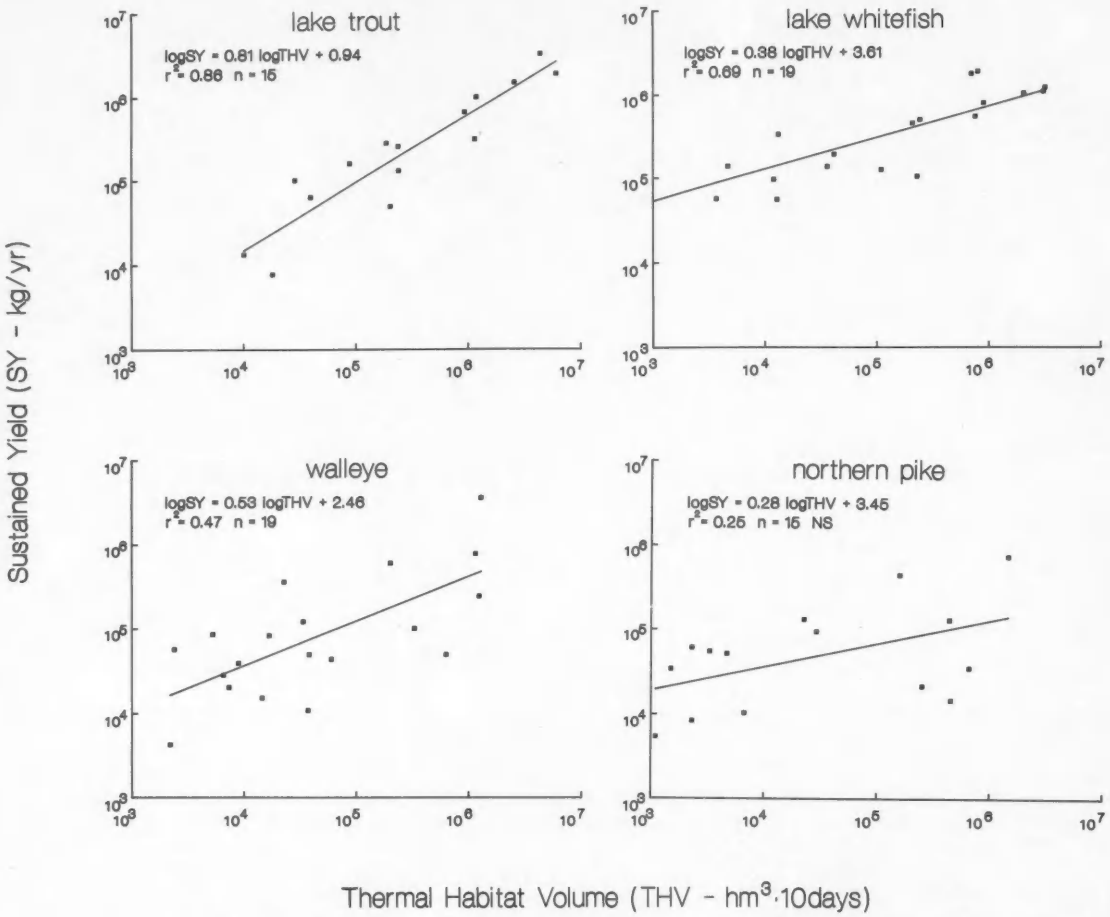
lake trout
logSY = 0.81 logTHV + 0.94
r² = 0.86 n = 15
lake whitefish
logSY = 0.38 logTHV + 3.61
r² = 0.69 n = 19
walleye
logSY = 0.53 logTHV + 2.46
r² = 0.47 n = 19
northern pike
logSY = 0.28 logTHV + 3.45
r² = 0.25 n = 15 NS
Sustained Yield (SY - kg/yr)
Thermal Habitat Volume (THV - hm³·10days)

FIG. 5. Plots of total sustained yield versus thermal habitat volume for the four species.

addition of THAI increases the variation explained in the pike and walleye SY by 55.1 and 57.8%, respectively, over simple area.

Simple correlation analyses including all the variables in Table 1 showed THA and THV to be the only significant correlates of SY for walleye and northern pike (Christie 1986); THA is the better in both cases (Table 8). For lake trout, THV and THA were the best correlates of SY; area and volume are also significant correlates as shown above (Table 8). In addition, mean depth ($r = 0.852$) and MEI ($r = -0.783$) are significantly correlated with lake trout SY. As shown, lake whitefish SY is more closely correlated with area and volume than with THV or THA (Table 8). Mean depth ($r = 0.799$) and MEI ($r = -0.638$) are also significantly correlated with whitefish SY.

The analyses were extended with the addition of the edaphic measure TDS to the simple regressions of the absolute thermal habitat measures (THA and THV) (see Table 9). The best results of addition of the TDS term to the regression equations are reported here (see Christie (1986) for more complete details).

The addition of the TDS term to the THV equation for lake trout yield improves its predictive power (4.5%). The negative coefficient for the TDS term indicates that clear, less productive systems are more suitable to lake trout production (see Schlesinger and Regier 1983). TDS adds considerably to the predictive capacity of the THV equation for whitefish SY (8.2%) and to the simple volume equation (4.3%). The positive coefficients for TDS in these equations suggest that more fertile systems produce more whitefish, in contrast with the response of lake trout. While there is, surprisingly, no improvement to the prediction of walleye SY with the addition of the TDS term to the THA equation, there is an improvement for pike (6.0%).

Discussion

The strong relationships between the specific thermal habitat measures and total SY of particular species support the hypothesis that environmental temperature affects the production of individual species. More specifically, the availability, during the summer period, of water of a temperature range which is physiologically optimal for a species contributes strongly to the productive capacity of that species' population in a given lake. The results suggest that the application of the final preferendum (Fry 1947), a key measure of a species' thermal autecology, as a niche dimension in the fundamental thermal niche concept of Magnuson et al. (1979), allows for the successful characteri-

TABLE 7. Regression equations relating species total sustained yields (SY, kg/yr) to lake surface area (ha) and total lake volume (hm^3). The number of lakes examined in the relationship (n) and the coefficient of determination (r^2) are presented. Regressions are significant (F-test, $p << 0.01$) except as noted.

Lake trout	$\log_{10}$ SY = 0.933 $\log_{10}$ area − 0.111 n = 15, r^2 = 0.706
	$\log_{10}$ SY = 0.645 $\log_{10}$ volume + 1.890 n = 15, r^2 = 0.758
Lake whitefish	$\log_{10}$ SY = 0.540 $\log_{10}$ area + 2.462 n = 19, r^2 = 0.753
	$\log_{10}$ SY = 0.370 $\log_{10}$ volume + 3.647 n = 19, r^2 = 0.761
Walleye	$\log_{10}$ SY = 0.322 $\log_{10}$ area + 3.097 n = 19, r^2 = 0.148, NS, $p > 0.05$
	$\log_{10}$ SY = 0.197 $\log_{10}$ volume + 3.913 n = 19, r^2 = 0.117, NS, $p > 0.05$
Northern pike	$\log_{10}$ SY = 0.137 $\log_{10}$ area + 3.992 n = 15, r^2 = 0.044, NS, $p > 0.05$
	$\log_{10}$ SY = 0.039 $\log_{10}$ volume + 4.488 n = 15, r^2 = 0.008, NS, $p > 0.05$

zation of the thermal environment available to fish. The results indicate a clear connection between the effect of temperature on the organismal response of fish growth and metabolism and the population response of production and yield.

The relationship between SY and THA for northern pike has the smallest r^2 found for the four species. The SY estimates for pike may be less accurate than for the other three species. Pike, because of low market value and its importance as a sport species, has not been a prime commercial species. Its relative importance to the total commercial catch may be greater within the various inland lakes than in the Great Lakes.

The spatial orientation of each species to the bottom or pelagium of the lake is important to the interpretation of the thermal habitat measures. For a benthic species, which orients to the bottom for food or shelter requirements, the bottom area within its optimal temperature range at any given time is important. For a pelagic species which exploits the food and space available off the bottom, the volume of water within its optimal temperature range will be important.

Northern pike orient to structure, feeding within macrophyte beds and other camouflage, so they can be classified as benthic. Walleye also orient to the bottom and its structure, both for sanctuary and feeding. Benthic invertebrates are the most important component in the diet of lake whitefish, but they may feed on plankton in some instances. Thus, while whitefish may move vertically off the bottom, their basic feeding orientation is to the bottom. Lake trout exploit food resources throughout the water mass; their food includes benthic invertebrates and fish and pelagic plankton and fish. These a priori distinctions imply that THA would be most applicable to pike, walleye, and whitefish and that THV would be more appropriate for lake trout. Our statistical results support these generalizations.

The results of addition of the edaphic variable TDS to the relationships suggest that these thermal habitat measures could be used to develop strong predictive multivariate equations for SY. Using these thermal habitat measures as limits of effective space available in lakes, the response of species yields and production could be examined with respect to the many other driving variables which have been explored in the literature (e.g. primary productivity (Oglesby 1977) or macrobenthos (Matuszek 1978; Hanson and Leggett 1982)).

We emphasize that this has been an exploratory study. Certain statistical relationships, say between yield and depth or TDS, should not be extrapolated beyond the ranges of the underlying data because the causal interconnections between the variables have not been well clarified. For example, SY values for lake trout were found to be positively correlated with mean depth and negatively with TDS: if extrapolated beyond reason, these findings would seem to imply that yields would be maximal in bottomless lakes filled with distilled water. Our relationships between yields and measures of optimal thermal habitat are based on some causal understanding of how species' ecological niches are determined in part by physiological optima. If these relationships survive further testing, then extrapolation beyond the present data should not generate nonsense.

Our findings might be tested against life history parameters of species. Patterns in growth rates and mortality rates with air temperature have been established by Pauly (1981) and Colby and Nepszy (1981); these might be explored using the measures of environmental temperature developed here. Year-class strengths have been associated with critical temperature events for a number of species, e.g. smallmouth bass (*Micropterus dolomieui*) (Shuter et al. 1980) and lake whitefish (Christie and Regier 1973). Our measures, applied on a year-specific rather than in an averaged manner, may have relevance to year-to-year population success of some species.

The relationship of SY of species to these thermal habitat measures suggests relevance to the assessment of the impacts of climate change impact. Connections between water temperature regime and atmospheric variables developed by physical limnologists may be examined with respect to the measures of specific thermal habitat space found in lakes. Once these con-

TABLE 8. Coefficients of determination (r^2) for the regressions of species sustained yield (SY, kg/yr) values on several independent variables ($\log_{10}$-transformed values). See text for variable definitions and units. NS indicates that the regression is not significant (F-test, $p > 0.05$).

		Independent variable					
Species	Number of lakes	THA	THV	Area	Volume	Area, THAI	Volume, THVI
Lake trout	15	0.811	0.859	0.706	0.758	0.811	0.908
Lake whitefish	19	0.658	0.692	0.753	0.761	0.757	0.762
Walleye	19	0.724	0.525	0.148 (NS)	0.117 (NS)	0.726	0.695
Northern pike	15	0.522	0.277 (NS)	0.044 (NS)	0.008 (NS)	0.595	0.526

TABLE 9. Best multiple regressions found for species' total sustained yields (SY, kg/yr) with the thermal habitat measures or lake size measures and the concentration of total dissolved solids (TDS, mg/L), a measure of lake fertility. The number of lakes examined in the relationship (n) and the coefficient of determination (r^2) are presented. All regressions are significant (F-test, $p << 0.01$).

Lake trout	$\log_{10}$ SY = 0.865 $\log_{10}$ THV − 0.693 $\log_{10}$ TDS + 1.967 n = 15, r^2 = 0.904
Lake whitefish	$\log_{10}$ SY = 0.397 $\log_{10}$ THV + 0.736 $\log_{10}$ TDS + 2.023 n = 19, r^2 = 0.774 $\log_{10}$ SY = 0.377 $\log_{10}$ volume + 0.528 $\log_{10}$ TDS + 2.557 n = 19, r^2 = 0.804
Walleye	$\log_{10}$ SY = 0.872 $\log_{10}$ THA − 0.077 $\log_{10}$ TDS + 0.525 n = 19, r^2 = 0.724
Northern pike	$\log_{10}$ SY = 0.530 $\log_{10}$ THA + 1.003 $\log_{10}$ TDS + 0.012 n = 15, r^2 = 0.582

nections are made, the relationships described here could be used to predict first-order population effects of climate change (Meisner et al. 1987).

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Allometric Models of Simuliid Growth Rates and Their Use for Estimation of Production

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Growth rates of *Prosimulium mixtum/fuscum*, *Stegopterna mutata*, and *Simulium vittatum* are described in relation to individual mass, water temperature, and seston quality in the outlet of Lake Orford (southern Quebec) in the winter of 1985–86. Growth rates in mass (G, per day) of the three species were similar and inversely related to dry mass (DM, micrograms) following the power relationship $G = 0.11\ DM^{-0.28}$; neither water temperature nor seston abundance and quality varied sufficiently during the study period to have a significant influence on growth rates. The allometric model was used to estimate production of the three species, and the resulting estimates are compared with cohort methods and size–frequency estimates. Large larvae were responsible for most of the production, and most of the production occurred before the spring rise in water temperature. Measured growth rates in winter are significantly lower than those reported for simuliids in warmer waters. A reanalysis of published data shows that growth rates of simuliids increase as the square root of water temperature (T, degrees Celsius) ($G = 0.08\ DM^{-0.21}\ T^{0.48}$).

On décrit les taux de croissance de *Prosimulium mixtum/fuscum*, *Stegopterna mutata* et *Simulium vittatum* par rapport à la masse individuelle, la température de l'eau et la qualité du seston dans l'émissaire du lac Orford (sud du Québec) au cours de l'hiver 1985–1986. Les taux de croissance exprimés en masse (G, par jour) étaient semblables chez les trois espèces et étaient en relation inverse avec la masse sèche (DM, microgrammes) selon la relation de puissance $G = 0{,}11\ DM^{-0{,}m28}$. Ni la température de l'eau ni l'abondance et la qualité du seston ont suffisamment varié pendant la période expérimentale pour influer de façon significative sur les taux de croissance. On a utilisé un modèle d'allométrie pour estimer la production des trois espèces; les estimations ainsi calculées sont comparées à celles obtenues à l'aide des méthodes des cohortes et des estimations de la fréquence des tailles. Les grosses larves sont à la source de la grande partie de la production qui a eu lieu avant l'augmentation printannière de la température de l'eau. Les taux de croissance quantifiés en hiver sont nettement inférieures à ceux signalés chez des simuliidés fréquentant des eaux plus chaudes. Une nouvelle analyse de données publiées révèle que l'augmentation des taux de croissance des simuliidés est égale à la racine carrée de la température de l'eau (T, degrés Celsius) ($G = 0{,}08\ DM^{-0.21}\ T^{0.48}$).

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Precise and accurate estimates of secondary production of aquatic organisms are needed for rational management of freshwater resources and for the development and testing of ecological theories. Several calculation methods have been proposed to estimate production. Cohort methods (removal summation, increment summation, instantaneous growth, and Allen Curve) assume that the population has synchronous recruitment and can be applied only when the cohorts can be separated. However, many populations of invertebrates, especially those in warm waters, do not have discernible cohorts, and alternative methods (size–frequency, production/biomass method) must be used (Benke 1984; Benke and Jacobi 1986; Kimmerer 1987).

An ideal method to estimate production would retain all the information from the raw data. The size–frequency method, originally described by Hynes and Coleman (1968), and further improved by Hamilton (1969), Benke (1979), and Krueger and Martin (1980), estimates production from the reconstructed average annual size distribution of the organism. This method generally overestimates, if only slightly, the true value in computer simulations (Cushman et al. 1978) and obscures information about the timing of production over the sampling period. Benke and Jacobi (1986) and Kimmerer (1987) have proposed that growth rates, measured either in the field or in seminatural conditions, be used to estimate production. This approach estimates production for both the entire sampling period and each sampling interval and is therefore preferable to the size–frequency approach.

Theoretical and logistic considerations also influence the choice of a calculation method. Since growth rates of invertebrates are influenced by body size (Banse and Mosher 1980; Behmer and Hawkins 1986), temperature (Brittain 1976; Sweeney 1978), and food abundance and quality (Colbo and Porter 1979; Fuller and Mackay 1981), an assumed constant growth rate could bias estimates of production. On the other hand, field measurements of growth rates for each size class and for each

sampling interval require increased effort in the field, and the gains from such effort may be small. A potential compromise between accuracy and cost is to use allometric models to obtain realistic estimates of field growth rates.

Published models (Banse and Mosher 1980; Behmer and Hawkins 1986) describe the general patterns among taxa within a narrow range of relatively warm water temperature and may be exceedingly imprecise or inaccurate for particular cases. We here examine the effect of body size on the growth rates of three species of overwintering simuliids in a lake outlet, test whether variations in temperature and seston abundance and quality over the sampling period influenced the growth rates, utilize the resulting allometric model to estimate the production by the three species, and compare this estimate with those obtained by the size–frequency, increment summation, and instantaneous growth methods. To further generalize the allometric model of simuliid growth rate, we also compare our results with literature data and present a model including water temperature.

Materials and Methods

Study Site

The study was conducted in 1985–86 at the outlet of Lake Orford (45°30′N, 72°25′W) in the Eastern Townships of southern Quebec. Lake Orford is small (surface area = 1.22 km²), deep (mean depth = 17.7 m, maximum depth 48 m), and oligotrophic (mean spring phosphorus concentration = 6 μg/L, mean summer chlorophyll concentration = 3 μg/L) with a residence time of 2.3 yr. The stream is 3–5 m wide and runs over gravel, pebbles, and cobble for the first 95 m from the lake. The study site was a 25-m-long riffle located at 45 m from the lake. Annual average discharge is 300 L/s.

Sampling

The stream was sampled 11 times between October 1985 and April 1986. On each visit, water temperature was recorded and three 4.5-L plastic bottles were filled by immersion in the middle of the stream. Stream discharge was estimated from the cross-sectional area of the stream, and measurements of mean current velocity were obtained with a Pigmy current meter at 8–12 points across the stream. On eight sampling dates between 26 November 1985 and 18 April 1986, 12 rocks (diameter = 5–20 cm, total surface area = 75–225 cm²) were collected at random coordinates in the section. The rocks were immediately put in plastic bags and the samples preserved with 95% ethanol. Freezing temperatures prevented the use of a downstream net, but a preliminary study (Morin 1987) showed that loss of black fly larvae due to disturbance was minimal (maximum loss 3%).

Laboratory Methods

Water samples were analyzed for seston dry mass, seston ash-free dry mass, percent organic content, and chlorophyll concentration. Water samples were first filtered through a 250-μm sieve to remove large particles that are not ingested by black flies (Dinkel and Wenk 1976; Elsen 1979; Merritt et al. 1982). Seston dry mass was measured by filtering 1- to 1.5-L subsamples from each replicate water sample on preashed and preweighed Gelman A/E glass fiber filters (effective pore size = 1.2 μm). Dry mass (DM, milligrams per litre) was measured after drying 48 h at 60°C and cooling 2 h in a desiccator. Filters were then ashed again for 2 h at 485°C and ash-free dry mass (AFDM, milligrams per litre) of the seston estimated as the loss in mass on ignition. Organic content (OC, percent) was estimated as 100 (ash-free dry mass/total dry mass). Chlorophyll concentration (Chl, micrograms per litre) was determined spectrophotometrically, using the equation of Bergmann and Peters (1980), after 24 h of extraction in 95% ethanol. Periphyton biomass (PERI, milligrams chlorophyll per square metre) was estimated indirectly as the amount of chlorophyll on the rocks. Rock samples were preserved at collection time in a known volume of 95% ethanol. The preserved samples were kept on ice in a cooler for 24 h; a 12-mL aliquot of the alcohol was then centrifuged and treated like samples for sestonic chlorophyll. Rock surface areas were estimated from the mass of aluminum foil required to wrap them (Morin 1987). Periphyton biomass was expressed on an areal basis by dividing the amount of chlorophyll extracted by the total rock surface area.

Rocks were gently rinsed and cleaned over a 75-μm sieve; the larvae collected on the screen were then sorted and identified under microscope. Samples containing more than 200 larvae were subsampled with a Folsom plankton splitter. Larvae of *Prosimulium mixtum/fuscum* complex Syme and Davies, *Stegopterna mutata* (Malloch), and *Simulium vittatum* Zetterstedt were identified according to the criteria of Wood et al. (1963). Body length of each larva was measured to the nearest 0.1 mm with an ocular micrometer. The lengths were converted to dry mass with regression equations developed from larvae brought alive to the laboratory. These were immersed in 95% ethanol for 10 s to kill them and transferred to distilled water to be immediately measured, dried, and weighed. Densities and biomasses were expressed per square metre of rock surface.

TABLE 1. Dry mass (DM, μg) as a function of body length (L, mm) for three species of simuliids. The number of larvae used to estimate the regression (n) and coefficient of determination of the regression on log-transformed data (r^2) are also given.

Species	Equation	n	r^2
P. mixtum/fuscum	$DM = 1.359\ L^{3.047}$	144	0.95
S. mutata	$DM = 1.184\ L^{3.220}$	67	0.99
S. vittatum	$DM = 1.185\ L^{3.654}$	123	0.95

Statistical Methods

To estimate the length–mass regression equations for each species, the measured length and dry mass were first transformed to natural logarithms, and a linear regression was fitted to these transformed data. Regression equations were back-transformed to arithmetic units and corrected for the bias of the logarithmic transformation (Miller 1984; Bird and T.-Prairie 1985) (Table 1).

The size distributions of larvae of *P. mixtum/fuscum* and *S. mutata* were clearly bimodal for the early sampling dates, but the bimodality became less apparent by the end of the sampling season (see Fig. 1). For *S. vittatum*, there were three modes at the beginning of the season (November), but several smaller modes had appeared by February, confounding the separation of the cohorts. The objective separation of cohorts is simple when large numbers of larvae are collected and there is little overlap in the size or age distribution. When there is overlap in the size distributions of the cohorts, researchers usually rely on graphical interpretation of the size–frequency distributions (Cassie 1954; Harding 1949; Harris 1968; Southwood 1966; Taylor 1965) or on iterative algorithms (Akamine 1982, 1984;

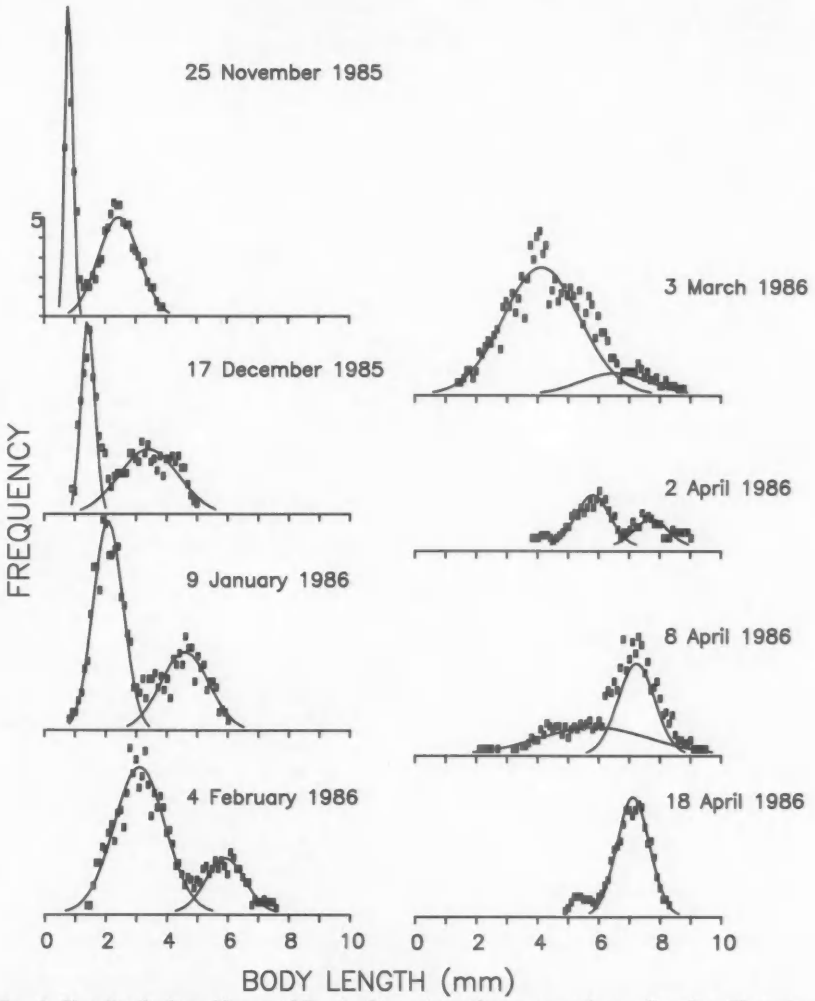

FIG. 1. Size distribution of larvae of *Prosimulium mixtum/fuscum* on each sampling date. The points are observed frequencies in 0.1-mm size classes (smoothed by 5-terms running average) and the lines represent the contour of the normal distributions fitted to the raw data.

Brassard and Correia 1977; Clark 1977; Macdonald 1969; Macdonald and Pitcher 1979) to divide the cohorts. We used the algorithm of Akamine (1982) to objectively separate the cohorts through the entire sampling season. The algorithm iteratively fits a predetermined number of normal distributions to a polymodal distribution from initial estimates of the parameters (mean, standard deviation, number of individuals in the distribution) that are provided by the user. Figure 1 illustrates the results by superimposing fitted distributions to observed frequencies of *P. mixtum/fuscum*. This method was preferred to graphical interpretation of the size distribution because it involves less subjectivity in the estimation of the parameters. The algorithm was iterated until convergence was attained (10–15 iterations). The resulting distributions were robust, since the same solution was obtained for different plausible starting points.

From the fitted distributions, we determined the mean mass of the larvae in each cohort on each sampling date by converting lengths to dry mass and calculating the mean dry mass weighted by the frequency of larvae in each size class. Daily growth rates were then determined for each cohort and each sampling interval using an equation that assumes exponential growth:

$$(1) \quad G = \ln (M_t / M_0)/t$$

where G is the instantaneous growth rate (per day), M_0 and M_t are the mean dry mass at the beginning and end of the sampling interval, and t is the time interval (days) between the two sampling dates. Growth rates were calculated for larvae of each cohort only for the interval covered by the first six sampling dates when cohort separation was most apparent and before large numbers of pupae were found.

Mean density and biomass of each species on each date were calculated as weighted averages, using individual rock surface area as the weight criterion. Appropriate transformations to stabilize the variance of density and biomass estimates were found by fitting mean–variance regressions (Downing 1979; Morin

TABLE 2. Regression equations relating daily growth rate (G, d^{-1}) to the individual dry mass (DM, μg) as log G = intercept + slope log DM. Standard error of the coefficients (SE), number of observations (n), and residual mean square (RMS) are also given.

Species	Intercept (SE)	Slope (SE)	n	r^2	RMS
P. mixtum/fuscum	−0.90 (0.10)	−0.31 (0.05)	10	0.84	0.011
S. mutata	−0.94 (0.08)	−0.29 (0.04)	10	0.86	0.007
S. vittatum	−1.00 (0.12)	−0.26 (0.05)	15	0.65	0.030
Combined	−0.96 (0.06)	−0.28 (0.03)	35	0.74	0.017

1985). Density of larvae in each cohort on each sampling date was determined from the relative surface area under each fitted distribution (e.g. Fig. 1).

Production estimates were calculated by the instantaneous growth, increment summation, and size–frequency methods as described in Benke (1984) and Krueger and Martin (1980). No correction for the cohort production interval (CPI) was necessary, since the sampling was done only during the productive period. Confidence intervals were calculated according to Newman and Martin (1983) for the instantaneous growth and increment summation estimates. For all confidence intervals, we assumed, following Krueger and Martin (1980), that the error in determination of mean mass was negligible compared with the error associated with estimates of mean density.

Production was also calculated by the instantaneous growth method from average biomass in each size class and the growth rates predicted from individual dry mass (Table 2):

$$(2)\quad P = \sum_{i=1}^{n} \sum_{j=1}^{m-1} 0.5(B_{ij} + B_{ij+1})G_i t$$

where n is the number of size classes, m is the number of sampling dates, B_{ij} is the biomass of larvae in size class i on sampling date j (milligrams dry mass per square metre), G_i is the daily growth rate of larvae in size class i (per day) as predicted from mean dry mass, and t is the number of days between sampling date j and $j + 1$.

Results

Temperature, Discharge, and Seston

Water temperature dropped rapidly in October and November and then remained below 2°C until the second week of April, slightly after the peak spring discharge (Fig. 2a). Discharge oscillated around the mean annual discharge (300 L/s) until the spring freshet and then dropped to the minimum observed value at the end of April. Peaks of discharge followed rains in October and November (see Fig. 3a) whereas a peak in February reflects snowmelt following a winter rainfall.

Seston ash-free dry mass remained essentially constant (≈0.4 mg AFDM/L) until the end of April and seston concentrations remained below 2 mg DM/L all winter, although significant changes were observed in April (Fig. 2b). Percent organic content of the seston varied from 20 to 65% over the sampling season, although the large errors associated with these measurements obscured any systematic trends (Fig. 2d). Percent organic content of the seston was inversely related to total seston concentration. The chlorophyll content of the seston generally dropped during the winter, except for a short period in the middle of the winter where it attained its maximum value. Periphyton biomass declined steadily between October and April (Fig. 2c), but variations in chlorophyll concentrations did not follow the variations in periphyton biomass, which suggests that most of the chlorophyll in the water came from the lake rather than from eroding periphyton.

Growth

Larval lengths of all three species increased linearly with time between November and the beginning of April (Fig. 3). The first cohort of *P. mixtum/fuscum* apparently hatched by the end of October and the second by the end of November (Fig. 3a). Pupae appeared in the stream by the beginning of April, and all larvae from the first cohort had apparently pupated by mid-April. *Stegopterna mutata* followed a similar pattern but the second cohort was relatively much more important than the first (Fig. 3b). In addition to the two fall cohorts, a third cohort of *S. vittatum* had apparently hatched in late summer or beginning of fall (Fig. 3c). Larvae of *S. vittatum* increased in length more slowly than larvae of the two other species. Pupae appeared in the stream as early as February, and by mid-April, all larvae of the earliest cohort had apparently pupated.

Growth rates decreased significantly with increasing individual mass (Fig. 4) but there were no significant differences among the three species (ANCOVA with species as treatments and individual mass as the covariate, $P > 0.5$; Table 2). The slower growth of *S. vittatum* in terms of body length was not reflected in growth rate in terms of dry mass because larvae of *S. vittatum* are sturdier than those of the two other species (exponent of the length–mass equation ≈3.7 vs. ≈3.1 for the other species). Once corrected for the effect of body size, growth rates were independent of water temperature, seston abundance, and seston quality (partial t-tests in multiple regression models, $P > 0.5$, except for chlorophyll concentration where $P = 0.12$).

Density, Biomass, and Production

Although larval biomass increased significantly from November to April (Fig. 5b), larval density did not change significantly until spring runoff (Fig. 5a), suggesting that mortality was low or offset by larval immigration. However, when larvae were assigned to their respective cohorts, it appeared that density of the early cohort of each species (cohort 1, the largest larvae) declined whereas density of larvae in the later cohorts (cohorts 2 and 3) increased or remained constant until spring runoff in April (Fig. 6).

Production estimates depended on estimation method and whether the calculations were made on individual cohorts or on the combined data (Table 3). The calculation method of Krueger and Martin (1980) for the size–frequency estimates yielded the highest estimates whereas the instantaneous growth estimate on combined cohorts yielded the lowest estimates. Instantaneous growth and increment summation estimates when cohorts were merged for the calculation were only 60–88% of the production estimates obtained by summing the production of each cohort. Approximate 95% confidence intervals (Newman and Martin 1983) were ±20–25%.

The estimates derived from the allometric model of growth rate and the biomass profiles on each sampling date were almost indentical to those obtained by the increment summation and instantaneous growth methods when applied to identified

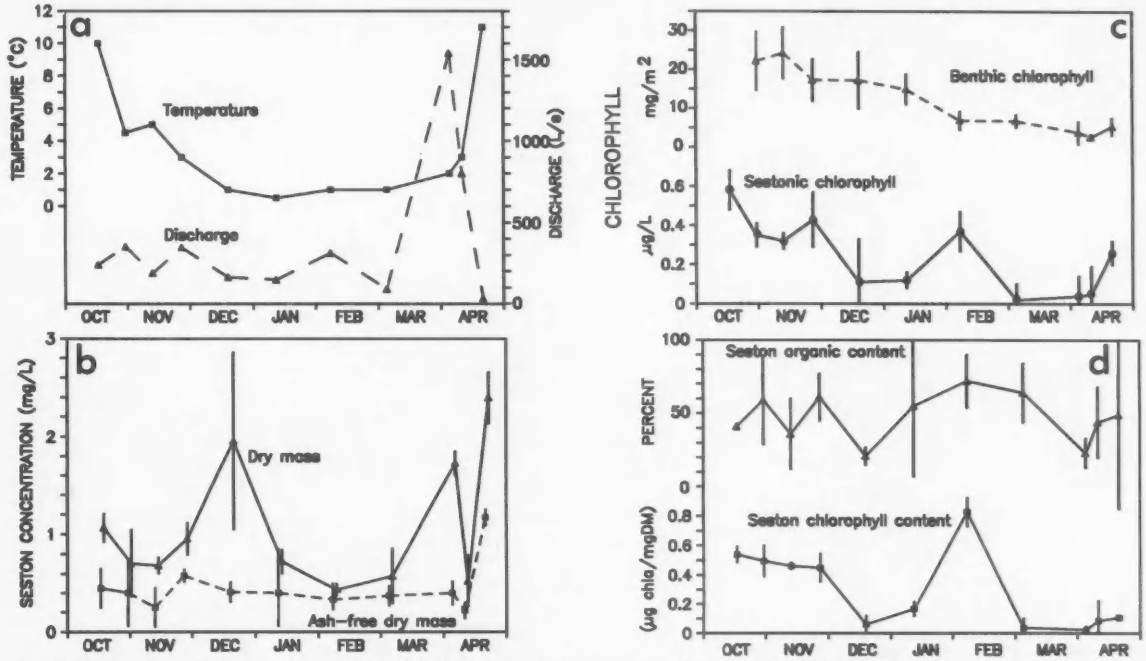

FIG. 2. Temperature, discharge, seston, and periphyton profiles for the winter of 1985–86 at the outlet of Lake Orford. Vertical bars represent ±2 SE of the means.

cohorts. The production estimate obtained by Eq. 2, like cohort methods, allows the estimation of production for each sampling intervals. Most of the production of the three species (between 50 and 70%, depending on species and the estimate of production used) occurred in February and March before the water temperature started to rise (Fig. 7a). Equation 2 also allows estimation of the contribution of each size class to the total production. Contribution of late instars was the most important, since larvae >4 mm long contributed 65–90% of total production of the three species (Fig. 7b).

Discussion

Population Dynamics

Larvae of *P mixtum/fuscum* and *S. mutata* generally hatch over several weeks in late fall or early winter when water temperature drops below 6–8°C (Davies and Peterson 1956; Ross and Merritt 1978; Colbo 1979). Ross and Merritt (1978) observed the hatch of a second cohort of these species in midwinter and related this to flooding of stream banks where eggs had survived. In the outlet of Lake Orford in 1985–86, these two species began to hatch in early October after a series of major rain events. A reduction of stream discharge in mid-October interrupted the hatch until the beginning of November when discharge rose again after another series of rain storms. This created two larval cohorts that grew through the winter. The same hypothesis would explain the presence of the two later cohorts of *S. vittatum* in addition to the cohort formed of older large larvae that did not complete their development before water temperature dropped.

Immigration of larvae in the studied section probably reflects the changes in their microhabitat requirements during growth. Cephalic head fans of first instar simuliids are rudimentary, and these larvae probably feed on periphyton and detritus. First instars of both *P. mixtum/fuscum* and *S. mutata* appear in the stream in late fall when periphyton biomass is high. After a short period feeding on this resource, they begin to filter-feed. This change in feeding strategy implies a relocation of the larvae where current velocities and turbulence are satisfactory for passive filtration and consequently a reduction of the size of the habitat that may support large larvae (Colbo 1979; Colbo and Moorhouse 1979).

This reduction of the usable habitat during growth can explain the observed variations in larval densities through the winter at our sampling station. Because adult females tend to oviposit at the mouth of pools or lakes, most first instar larvae hatch very close to the lake (Rubtsov 1964; Rühm 1970). If suitable filtering sites are limited, then the growing larvae must drift downstream in search of suitable habitat. Larvae that hatch later would find most suitable sites occupied by larger larvae and would be more likely to drift downstream. This would explain the increase in density of the youngest cohort from November to March. The increase in density could reflect recruitment of young larvae, but since no first instars were found after the beginning of January, this hypothesis would not explain the increase observed in February and March.

Production

The presence of several cohorts violates the assumptions underlying the estimation of production by cohort methods. Computer simulations (Morin et al. 1987) show that the increment summation and instantaneous growth estimates of production are robust to mild asynchrony, but tend to underestimate true production when asynchrony is large. In the

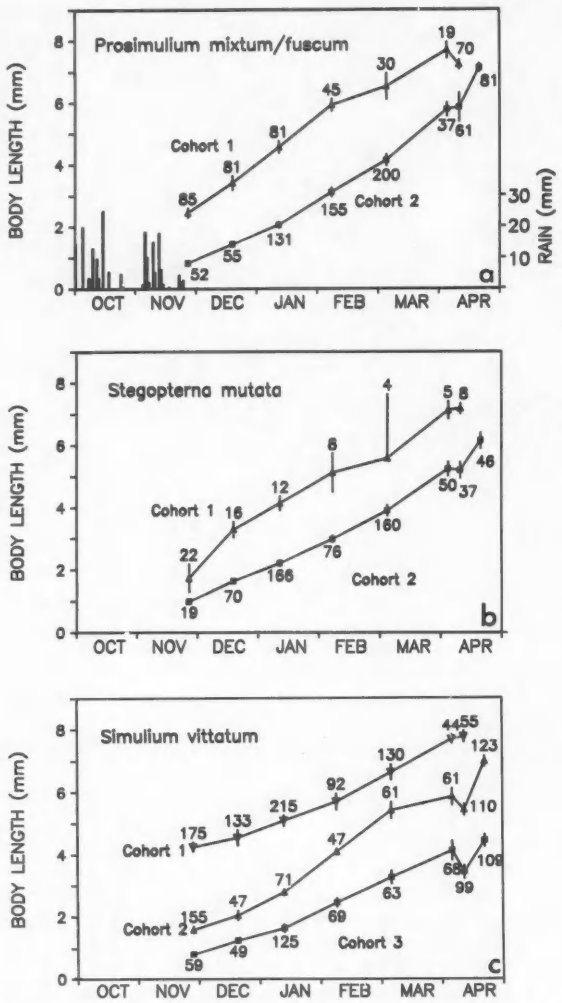

FIG. 3. Lengths of the larvae of each cohort. Vertical bars represent ±2 SE of the estimated means. Numbers are the number of measured larvae assigned to each cohort.

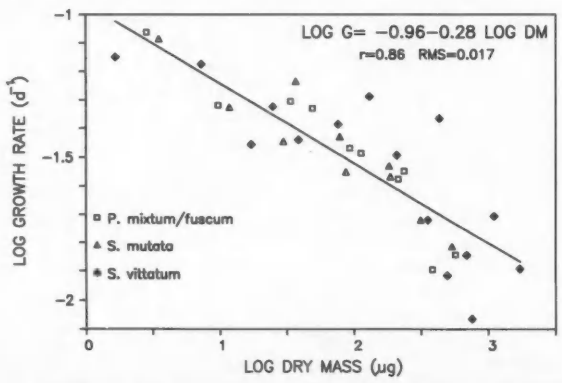

FIG. 4. Effect of dry mass of individual larvae on daily growth rates.

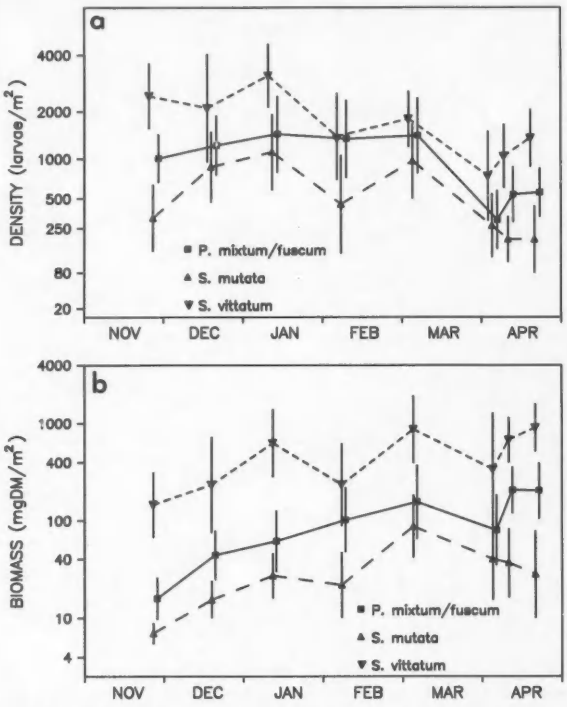

FIG. 5. Density (fourth root vertical scale) and biomass (logarithmic scale) of the three species on each sampling date.

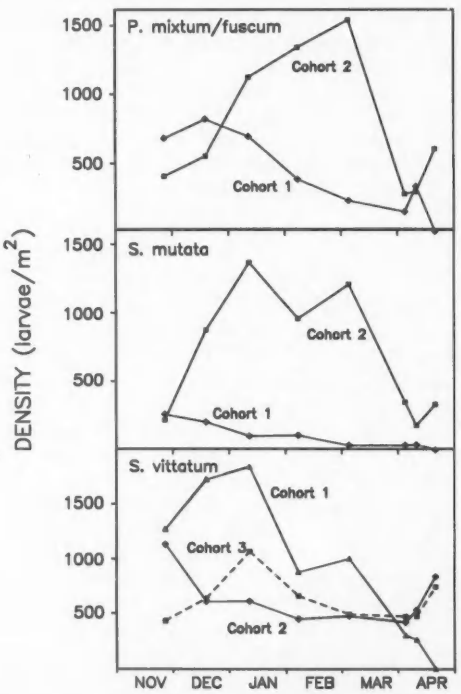

FIG. 6. Estimated density of larvae in each cohort on each sampling date.

TABLE 3. Secondary production estimates (mg DM/m²) for the three species using seven different methods.

Method	*P. mixtum/ fuscum*	*S. mutata*	*S. vittatum*
Size–frequency (Benke 1984)	799	359	5337
Size–frequency (Krueger and Martin 1980)	907	385	5441
Increment summation, cohorts combined (±95% CI)	575 (±120)	280 (±71)	1821 (±410)
Increment summation, sum of cohorts production	662	331	2931
Instantaneous growth, sum of cohorts production	633	302	2714
Instantaneous growth, cohorts combined (±95% CI)	524 (±125)	268 (±65)	1902 (±476)
Instantaneous growth (using mean observed biomass in 0.1-mm size classes on each date and growth rates predicted by the combined regression on dry mass of Table 2)	688	292	2566

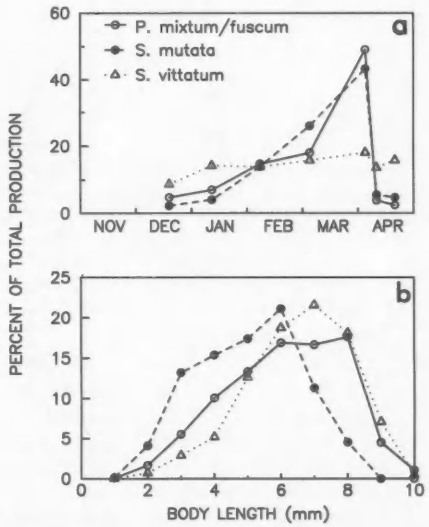

FIG. 7. Percent of total production by each species (a) during each sampling interval (b) made by 1-mm size classes.

populations studied here, it seems that the bimodal and trimodal recruitment led to underestimation of production when cohorts were combined. The size–frequency estimates for *S. vittatum* are much higher than those from all other methods and are probably biased because the sampling season only covered the last portion of the life span of the first cohort. The "average cohort" used in this analysis was therefore biased in favour of the highly productive large larvae. Since this population consisted of three cohorts, and cohort production interval differed among cohorts, it was not possible to correct the estimate appropriately. The size–frequency estimates for the two other species are also slightly higher than cohort method estimates, and this is probably because sampling should have started a month earlier to catch the hatch of the earliest cohort. By elimination, the best estimates of production are therefore those obtained by the increment summation and instantaneous growth methods of calculation applied to identified cohorts.

All calculation methods assume that larvae neither immigrate nor emigrate. Emigration of larvae is less of a problem, since it could be considered equivalent to mortality for a given area, but immigration of larvae would lead to an overestimation of production in the study area. Our data suggest that there was net immigration of larvae from upstream, and therefore, production is probably overestimated. Confidence intervals around the estimates may therefore not be meaningful (Hynes 1980) because they only reflect sampling variability of density and biomass on each date. This problem can be avoided either by monitoring immigration of larvae into the study reach, or by estimating population density for the entire area where larvae may be found. Without such estimates, production estimates and their confidence intervals should be considered only indicative.

For the three species in this study, the overestimation of production is probably small. Assuming a constant mortality rate of 1.25% per day (average mortality rate of the early cohort of each species), the biomass of larvae that immigrated into the study reach over the winter would have been 71, 51, and 283 mg DM/m² for *P. mixtum/fuscum*, *S. mutata*, and *S. vittatum*, respectively. The bias due to immigration would increase estimate of production to 110–130% of true production. The biases are apparently small because immigrating larvae are small, and the biomass of immigrating larvae is unimportant relative to production by larger larvae. In situations where most immigrating larvae are large (Colbo and Moorhouse 1979), the biases could be considerably larger.

The similarity of the production estimates obtained by cohort methods and those obtained from the biomass profile and predicted growth rates suggests that it is possible to reduce greatly the effort necessary to estimate production. Once the length–mass and growth rate–mass relationships are determined, it is neither necessary to separate cohorts nor to separate species with similar length–mass relationships. Since the growth rates vary with body size, the use of size-specific growth rates should yield estimates of production that are more accurate than the

TABLE 4. Effect of water temperature (T) and mean individual size (W, µg dry mass) of larvae on the predicted annual turnover ratio (P/B) of simuliid populations.

	T(°C)							
W (µg)	1	2	4	6	8	12	15	20
20	15	21	30	36	42	51	56	65
40	13	19	26	31	36	44	49	56
60	12	17	24	29	33	40	45	51
80	11	16	22	27	31	38	42	48
100	11	15	21	26	30	36	40	46
150	10	14	20	24	27	33	37	42
200	9	13	18	22	26	31	35	40
400	8	11	16	19	22	27	30	35

instantaneous growth estimates applied to pooled cohorts or to asynchronous populations.

Prediction of Growth Rates and Annual Turnover Ratios

Measured growth rates at the outlet of Lake Orford were not related to water temperature or seston quality. This may result from the small variability of these two factors during the sampling period. To further examine the effect of temperature, we collected literature data on simuliid growth rates at warmer temperatures.

Growth rates of larvae observed in this study were similar to growth rates reported in the literature for other species of simuliids at low (<4°C) temperatures, but significantly lower than those observed in warmer waters (Fig. 8a). To quantify the effect of temperature, we fitted a multiple regression model using individual mass and water temperature as the independent variables, yielding

$$(3)\quad G = 0.079\ \mathrm{DM}^{-0.21}\ T^{0.48} \qquad n = 70,\ R^2 = 0.76,\ \mathrm{RMS} = 0.031$$

where DM is the mean annual dry mass (micrograms) of individuals in the population, T is the water temperature (degrees Celsius), and RMS is the residual mean square of the regression in logarithmic form.

This model (Eq. 3) implies that daily growth rates rise approximately with the square root of water temperature. The model apparently fits adequately both sets of data (Fig. 8b), and this suggests that a single equation could be used for all simuliids. Since annual turnover ratios are 365 times the average daily growth rate of the population, the same equation could be used to predict annual production/biomass (P/B) ratios (Table 4).

Assuming that the cohort P/B ratio is a constant (5, Waters 1977), the cohort production interval (CPI (days), Benke 1984) could also be predicted from Eq. 3. Since the cohort P/B is the product of the average daily growth rate (G, per day) and the CPI,

$$(4)\quad \text{Cohort } P/B = G\ \mathrm{CPI},$$

the CPI can be estimated by

$$(5)\quad \mathrm{CPI} = (\text{cohort } P/B)/G = (5)/G.$$

Substituting G in Eq. 5 by the function described by Eq. 3 yields

$$(6)\quad \mathrm{CPI} = (5)/(0.079\ \mathrm{DM}^{-0.21}\ T^{0.48}).$$

Limits and Use of Allometric Models

Food availability and quality influence growth rates of simuliids (Carlsson et al. 1977; Colbo and Porter 1981; Schröder

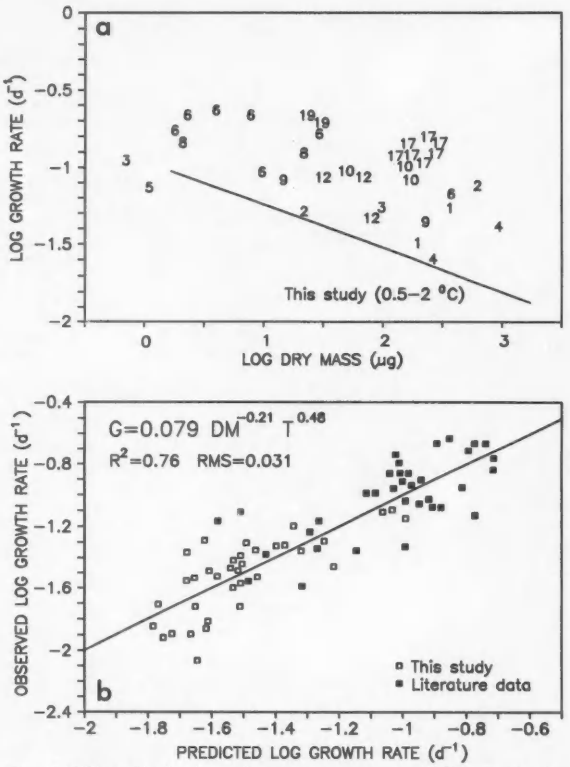

FIG. 8. Effect of body size and water temperature on daily growth rates of simuliid larvae. (a) Daily growth rates as a function of mean individual dry mass. Numbers are the reported water temperatures. (b) Observed growth rates and predicted values from the multiple regression model. Literature data from Carlsson et al. (1977), Wotton (1978), Merritt et al. (1982), Benke et al. (1984), and Behmer and Hawkins (1986).

1981). Difference in food supply may therefore explain some of the variability around the predicted values, and a better model of simuliid growth rates would include food quality or quantity. Such a model would provide researchers and managers with the required data to estimate accurately both total production and production per sampling intervals from biomass profiles on each sampling date.

Only indicative measures of uncertainty around the allometric estimates of production could be derived from the field data and the residual variability of the multiple regression model. The method will not allow the estimation of confidence intervals for the production estimate; only indicative confidence intervals could be obtained through Monte-Carlo simulations to combine the sampling error on biomass with expected variability around the regression model. However, the difference between the allometric estimates of production and the best cohort estimates (cohort methods applied to identified cohorts) was 5–10%, two to four times smaller than the width of the confidence intervals around cohort estimates (20–25%). This suggests that the potential bias of the allometric estimate of growth rate will often be hidden by the large sampling error associated with the estimates of density or biomass and that parametric or bootstrap confidence intervals considering only

sampling error on biomass would underestimate only slightly the uncertainty associated with the production estimate.

The multiple regression model predicting daily growth rate could be used to obtain production estimates from biomass profiles of simuliid populations when logistic support is limited or when the goal of the study is to describe large-scale patterns. The resulting estimates may not be as accurate as those that could be obtained by using species- and site-specific estimates of growth, but the method may be more appropriate for large-scale comparative studies, since it will allow a greater number of sites to be analyzed.

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Dissolved Carbohydrate Distribution and Dynamics in Two Southeastern United States Reservoirs[1]

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Alberts, J. J., J. W. Bowling, and M. Emmons. 1988. Dissolved carbohydrate distribution and dynamics in two southeastern United States reservoirs. Can. J. Fish. Aquat. Sci. 45: 325–332.

Dissolved carbohydrate concentrations were measured in the waters of two southeastern United States reservoirs (Pond B and Par Pond) in 1982 and 1983 with attention to the influence of rooted aquatic macrophytes, spatial and temporal variability, and depth. Mean concentrations for all sampling locations were 2.86 ± 1.01 mg·L^{-1} in Pond B and 1.72 ± 1.24 mg·L^{-1} in Par Pond. There were no consistent spatial or temporal differences within a reservoir, nor were there consistent differences between macrophyte beds and open waters in either impoundment. Dissolved carbohydrate concentrations increased in both reservoirs during the day, reaching maxima in late afternoon, with an accompanying decrease in the dissolved organic carbon (DOC). The dissolved carbohydrates comprised 5.8–20.2% of the DOC in Par Pond and 25.7–42.3% of the DOC in Pond B. In both reservoirs, vertical profiles show the presence of carbohydrates at all depths during aerobic and anaerobic conditions. Midwater concentration maxima were present in Par Pond during periods of holomixis and stratification. The maxima were coincident with an intense pycnocline during periods of stratification; but during holomixis, the maximum was apparently unsupported by physical structure.

En 1982 et 1983, on a quantifié les concentrations d'hydrate de carbone dissous dans les eaux de deux réservoirs du sud-est des États-Unis (étangs B et Par) en accordant une attention particulière à l'influence des macrophytes aquatiques fixés, à la variabilité spatiale et temporelle et à la profondeur. Pour tous les sites d'échantillonnage, les concentrations moyennes s'élevaient à 2,86 ± 1,01 mg·L^{-1} dans l'étang B et à 1,72 ± 1,24 mg·L^{-1} dans l'étang Par. Il n'y avait pas de différences spatiale ou temporelle uniformes à l'intérieur d'un réservoir, ni entre les gisements de macrophyte et les eaux libres dans les deux bassins. Les concentrations d'hydrate de carbone dissous ont augmenté pendant la journée dans les deux réservoirs pour atteindre un maximum vers la fin de l'après-midi; cette augmentation était accompagnée d'une diminution de la concentration de carbone organique dissous (COD). Les hydrates de carbone dissous constituaient de 5,8 à 20,2 % de la COD dans l'étang Par et de 25,7 à 42,3 % de la COD dans l'étang B. Dans les deux réservoirs, les profils verticaux révèlent la présence d'hydrates de carbone à toutes les profondeurs en présence de conditions aérobie et anaérobie. On a observé des concentrations maximum dans les eaux centrales de l'étang Par au cours de périodes d'holomixie et de stratification. Les maxima coïncidaient avec un pycnocline prononcé pendant les périodes de stratification; toutefois, durant l'holomixie, le maximum n'était pas en apparence soutenu par la structure physique.

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Carbohydrates are an important fraction of the dissolved organic carbon (DOC) content of surface waters. Yet, relatively little is known of the spatial and temporal changes of this group of compounds in lake waters (Walsh 1965a, Thurman 1985). We are unaware of any published studies of dissolved carbohydrate distributions and/or dynamics in southeastern United States aquatic systems.

Below, we report the results of a study of the distribution of total dissolved carbohydrates in two southeastern United States reservoirs, with respect to spatial variation, depth distribution, anoxic versus aerobic water conditions, temporal and diurnal variability, effect of rooted aquatic macrophyte communities, and contribution to the total DOC pool.

Study Sites

The two reservoirs are located on the Savannah River Plant, Aiken, Allendale, and Barnwell counties, South Carolina (Fig. 1). The two systems, Pond B and Par Pond, have been described

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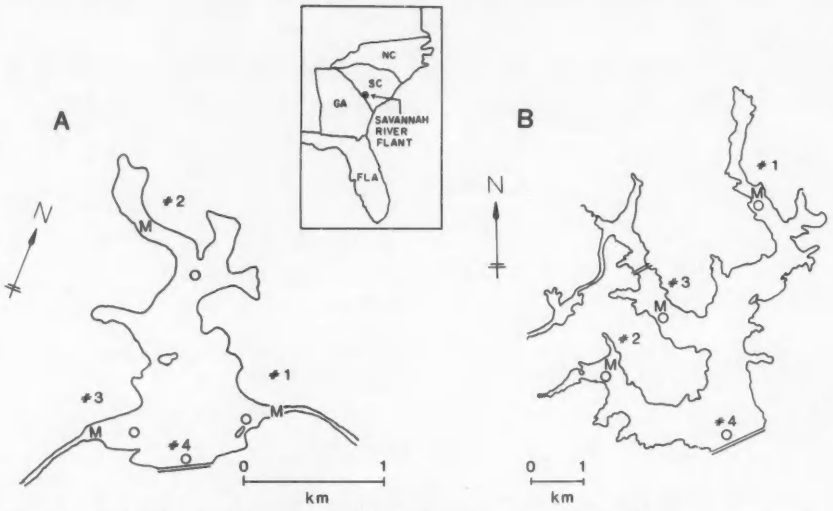

FIG. 1. Station locations in (A) Pond B and (B) Par Pond. O = open-water stations; M = macrophyte beds. Station 4 is the deepest station in both ponds.

previously (Parker et al. 1973; Tilly 1975; Giesy and Tessier 1979; Evans et al. 1983; Wilde 1983; Alberts and Dickson 1985). These impoundments lie within 2 mi (3.2 km) of each other and were created in the early 1960's as cooling water reservoirs for nuclear reactors. Although once having a common water supply from the Savannah River, they have been separated since 1964. Par Pond still receives thermal effluent from reactor operations and additional water inputs from the Savannah River which drains the Piedmont region of north Georgia, South Carolina, and North Carolina. The dissolved solids concentrations have slowly increased due to evaporation of water (Tilly 1975). Pond B has not received thermal effluents or river water inputs since the time of its isolation from Par Pond. It remains a softwater, slightly acid system with the primary freshwater inputs being rainwater and perhaps percolation of groundwater (Wilde 1983; Alberts and Dickson 1985). Both still undergo intense thermal stratification with anoxic hypolimnia for approximately 6 mo each year (Alberts et al. 1987).

Brasenia scherberi, Cabomba caroliniana, Nymphea odorata, and *Nymphoides cordata* were responsible for approximately 70% of the total dry biomass of rooted macrophytes in Pond B (F. W. Whicker, pers. comm.). In thermally unaltered regions of Par Pond, *Myriophyllum spicatum* and *Eleocharis acicularis* comprised approximately 90% of the plant biomass whereas if temperatures are elevated due to reactor cooling water inputs, *M. spicatum* and *E. acicularis* comprised 64% of the biomass, with *Najas guadalupensis* and *Potomogeton pusillus* becoming significantly more important components of the macrophyte biomass (Grace and Tilly 1976; Grace 1977). Total standing crop of phytoplankton in Pond B is lower than in Par Pond (mean = 1316 orgs·mL^{-1}, range = 165–2281 orgs·mL^{-1}; mean = 3779 orgs·mL^{-1}, range = 556–8456 orgs·mL^{-1}, respectively).

Methods

Carbohydrate concentrations were determined by the anthrone colorimetric technique using a glucose standard (Golterman et al. 1978). All glassware was soaked overnight in 10% KOH and then dry ashed at 450°C for several hours. Analyses were performed on a Beckman Instruments model Acta CIII ultraviolet–visible spectrophotometer.

TABLE 1. Carbohydrate concentrations (mg·L^{-1}) in open surface water (OW) and bordering macrophyte beds (MB) of two ponds. When reported, errors are ±1 SD of two replicate samples; ND = not determined.

	Pond B			
	22 July 1982 (1130)	27 July 1982 (1330)	4 Apr. 1983 (1200)	9 May 1983 (1000)
1 OW	2.75 ± 0.00	4.52	3.51	2.16 ± 0.72
1 MB	2.14 ± 0.27	5.00	1.80	2.58 ± 0.42
2 OW	3.00 ± 0.28	3.47	4.08	2.30 ± 0.48
2 MB	3.20 ± 0.14	5.96	2.94	3.83 ± 0.42
3 OW	2.50 ± 0.07	2.56	4.08	1.60 ± 0.64
3 MB	3.79 ± 0.23	2.96	3.51	2.30 ± 0.24
4 OW	2.55	2.50 ± 0.28	3.51	ND
	Par Pond			
	20 July 1982 (1530)	26 July 1982 (1130)	4 Apr. 1983 (1530)	9 May 1983 (1430)
1 OW	4.38 ± 0.74	2.23 ± 0.67	1.23	1.05 ± 0.64
1 MB	6.53 ± 1.52	2.00 ± 0.00	1.23	1.60 ± 0.24
2 OW	3.55 ± 0.07	1.48 ± 0.21	0.66	0.91 ± 0.72
2 MB	4.65 ± 0.21	1.83 ± 0.11	1.80	2.16 ± 0.42
3 OW	4.38 ± 1.73	4.25	0.66	1.02 ± 0.91
3 MB	3.45 ± 0.35	1.33	0.66	0.35 ± 0.24
4 OW	3.30	1.57 ± 0.09	1.23	ND

Surface water samples (250–500 mL) were collected by immersing clean polyethylene bottles below the surface of the water. Depth profile samples were obtained by pumping (Masterflex model pump, ColeParmer, Inc.). Samples were kept on ice and returned to the laboratory for filtration through prewashed, 0.45-μm membrane filters (Millipore Corp.). Appropriate blanks were analyzed to check for potential contamination from the filters. Aliquots of filtered water samples were placed in KOH-soaked bottles and stored frozen before analysis for DOC (Beckman model 915 carbon analyser). Physico-

chemical parameters were determined with a model 8000 in situ water analyser (Hydrolab Corp.).

Four sampling stations were established in each reservoir (Fig. 1). Stations 1–3 had two sampling sites, one along the shoreline in the macrophyte beds (M) and another in the open water (O) of the embayment. The fourth station (4) was over the deepest portion of each reservoir and had no macrophyte site. Samples for temporal and spatial variation in carbohydrate concentration were collected at all four stations on four occasions (20–22 and 26–27 July 1982, 4 April 1983, and 5 May 1983). On three occasions (4–5 August 1982, 4 April 1983, and 11 August 1983), depth profiles for dissolved carbohydrates and associated physico-chemical parameters were taken at Station 4. Samples for diurnal changes in dissolved carbohydrates were taken in the macrophyte beds and open-water sites of Station 3 in each reservoir. Samples were collected in alternate hours starting at 0600 in Par Pond and ending at 1900 in Pond B.

Results

On the four sampling days in the summer of 1982 and spring of 1983, the concentrations of carbohydrates in the <0.45 μm fraction of surface waters ranged from 1.60 to 5.96 $mg \cdot L^{-1}$ in Pond B and from 0.66 to 6.53 $mg \cdot L^{-1}$ in Par Pond (Table 1). With the exception of the first sampling period, Par Pond had lower concentrations of carbohydrates than Pond B. However, there were no consistent temporal or spatial differences in carbohydrate concentrations common to both systems, nor were there consistent differences in the carbohydrate concentrations between open water and adjacent macrophyte beds.

Both reservoirs were thermally stratified with anoxic hypolimnia during August of 1982 and 1983 (Fig. 2). The thermocline in Par Pond was at 5–6 m on both occasions, while in Pond B, it was at 4–5 m in 1982 and at 3–4 m in 1983. The increased conductivity in the deep water of both reservoirs is indicative of the flux of both inorganic and organic material out of the sediments as a result of the anaerobic conditions (Marshall and LeRoy 1971; Evans et al. 1983; Alberts et al. 1987, 1988). Holomixis was observed in Par Pond on 4 April 1983. Measurable oxygen concentrations occurred well into the deep waters of Pond B on this date, but oxygen depletion was observed in the deepest waters as thermal stratification had begun.

A maximum in carbohydrate concentrations in Par Pond occurred at 5–6 m during all three sampling occasions, despite the differences in the water column structure (Fig. 2). A minimum value in carbohydrate concentrations occurred in Pond B at 8–10 m of depth on all three sampling occasions. During both August sampling periods, the carbohydrate concentrations of the near-bottom water in Pond B increased over the minimum values seen at 8–9 m. A similar increase in deepwater carbohydrate concentration was observed in Par Pond during August 1983.

The data from the diurnal study (Fig. 3) again indicated that carbohydrate concentrations in Pond B were greater than those in Par Pond. Once more, there was no difference in the carbohydrate concentrations of the open waters and macrophyte beds during the morning hours in either reservoir. However, carbohydrate concentrations increase in both reservoirs after noon with a peak between 1600 and 1700. During this period, the concentration of dissolved carbohydrates in the macrophyte beds was higher than those observed in the open waters.

The DOC concentrations (Fig. 3) did not mirror the dissolved carbohydrate concentrations. In both reservoirs, the DOC values were higher in the early morning and declined toward afternoon. Extreme variations in some of the DOC values, particularly at 1030 in Par Pond, complicated the interpretation of the DOC trends. However, low DOC values at noon at both open water and macrophyte beds of Par Pond confirmed a general downward trend in this parameter. DOC concentrations did not differ between open waters and macrophyte beds.

Discussion

Spatial and Temporal Carbohydrate Distributions

The DOC pool of these reservoirs may contain as much as 50% of the DOC as humic acids (Alberts and Dickson 1985). Several investigators have reported that the anthrone method (Stabel 1977; Ochiai and Ukiya 1981) and the orcinol – sulfuric acid method (deHaan and deBoer 1979) overestimate carbohydrate concentrations in the presence of humic and fulvic acid materials. We added humic acid (Aldrich Chemical Co.) to Par Pond water, and following hydrolysis, the samples were analysed by the anthrone method. Concentrations of humic acids at 5 mg $C \cdot L^{-1}$ only increased the absorbance readings by 0.005 absorbance unit. Thus, if 50% of the DOC in the pond waters was humic acid, we would only expect a maximum correction in the carbohydrate concentration of 0.15–0.30 $mg \cdot L^{-1}$.

The range of dissolved carbohydrate concentrations in lake waters has been reported to be between 0.1 and 5.0 $mg \cdot L^{-1}$ (Thurman 1985; Steinberg and Münster 1985). The concentrations of dissolved carbohydrates reported for the two reservoirs in this study ranged from 0.7 to approximately 5.0 $mg \cdot L^{-1}$, with two isolated values between 6.0 and 6.5 $mg \cdot L^{-1}$, for a total of 215 individual carbohydrate samples. The average carbohydrate concentrations of Pond B and Par Pond for all seasons and depths were 2.86 ± 1.01 and 1.72 ± 1.24 $mg \cdot L^{-1}$, respectively (Table 2). Within the same pond, mean carbohydrate concentrations differed little between open waters and macrophyte beds. Mean concentrations of dissolved carbohydrates in Par Pond appear to be lower than those in Pond B, but in all cases the variability makes generalizations difficult.

High variability in dissolved carbohydrate concentrations is not unexpected when data from several sampling periods and seasons are averaged. Harvey (1983) reported large variations in the dissolved carbohydrate concentrations in the New York Bight for hourly, daily, weekly, and monthly samples. Several other investigators have reported large variations in lake waters on a seasonal basis, with highest concentrations usually occurring in the winter months (Walsh 1966; Berman et al. 1979; deHann and deBoer 1979; Ochiai et al. 1979; Ochiai et al. 1980a; Ochiai and Ukiya 1981; Steinberg and Münster 1985).

Diurnal Carbohydrate Trends

Hourly trends of the DOC and dissolved carbohydrate generally show a correspondence between open waters and macrophyte beds in both reservoirs (Fig. 3). There is an increase in dissolved carbohydrate concentration in the macrophyte beds during late afternoon in both ponds, but it appears to be of short duration. In addition, there is a general decline in DOC concentrations from morning to evening.

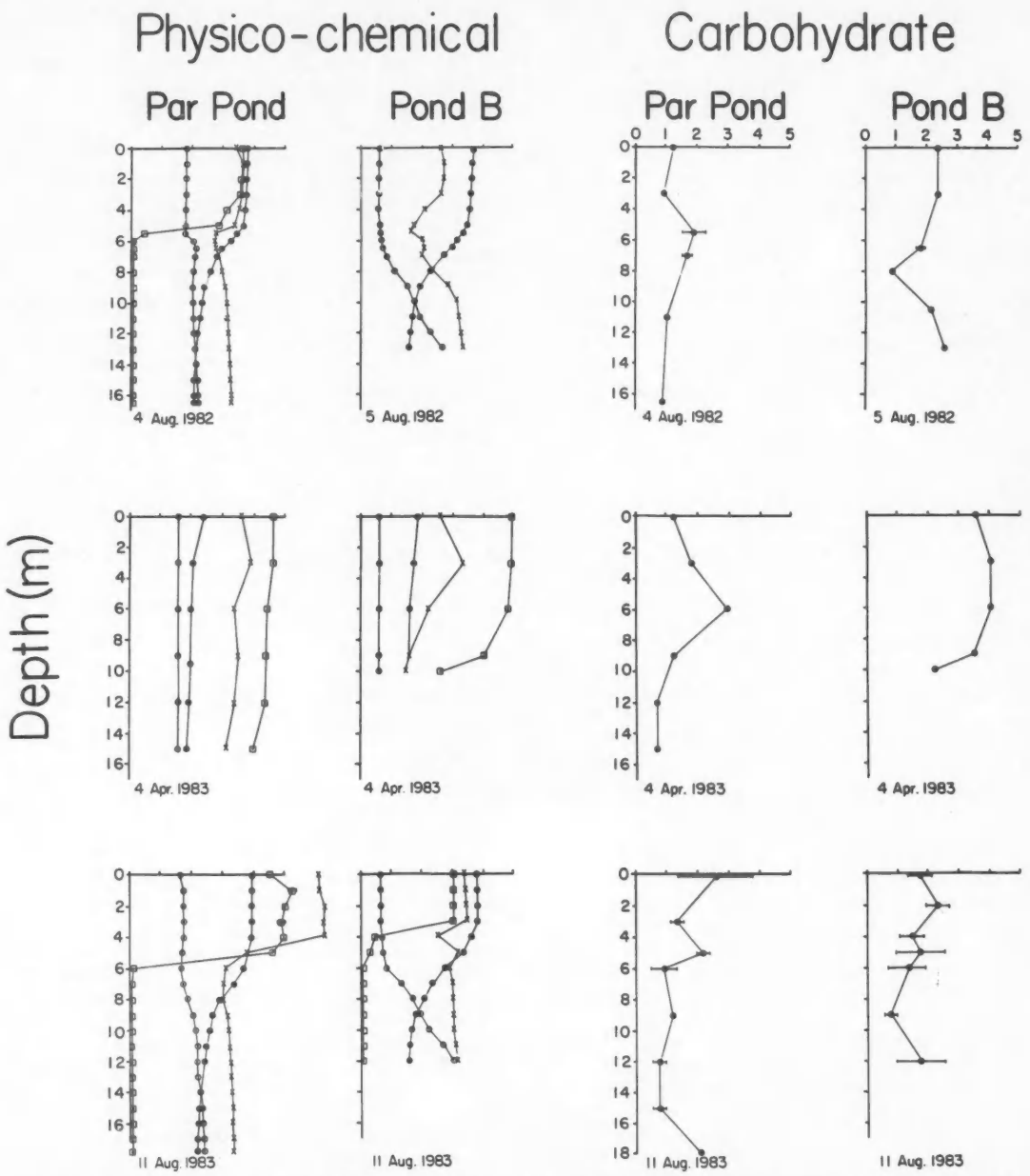

FIG. 2. Depth distributions of physico-chemical parameters and dissolved carbohydrate concentrations (mg C·L^{-1} ± 1 SD) at Station 4 in Par Pond and Pond B for 4 August 1982, 5 August 1982, 4 April 1983, and 11 August 1983. Temperature: ●, 0–40°C; pH: ×, 4–8 units; dissolved oxygen: □, 0–10 ppm; conductivity: ○, 0–200 μS; microsiemens. Actual data can be obtained by writing the senior author.

A similar pattern of increased dissolved carbohydrate concentrations during the day has been observed in other ponds. Walsh (1965b) concluded that the diurnal carbohydrate cycle was a function of phytoplankton density and light intensity. Münster (1984) concluded that in surface waters the total mono- and disaccharides followed the diel periodicity of production and respiration in the microbial community. The similarity of concentrations of dissolved carbohydrates in the open water and macrophyte beds observed in this work would also argue for microbial rather than macrophytic control.

Aquatic macrophytes can contain as much as 20% monosaccharides by dry weight (Rogers 1965; Boyd 1969; Mutzar et al. 1979) and can excrete DOC (Wetzel 1969a) that can comprise a significant portion of the photosynthetically fixed carbon in calcareous lakes (Wetzel 1969b). Furthermore, the study of *Najas flexilis* in calcareous lakes demonstrated the dependence

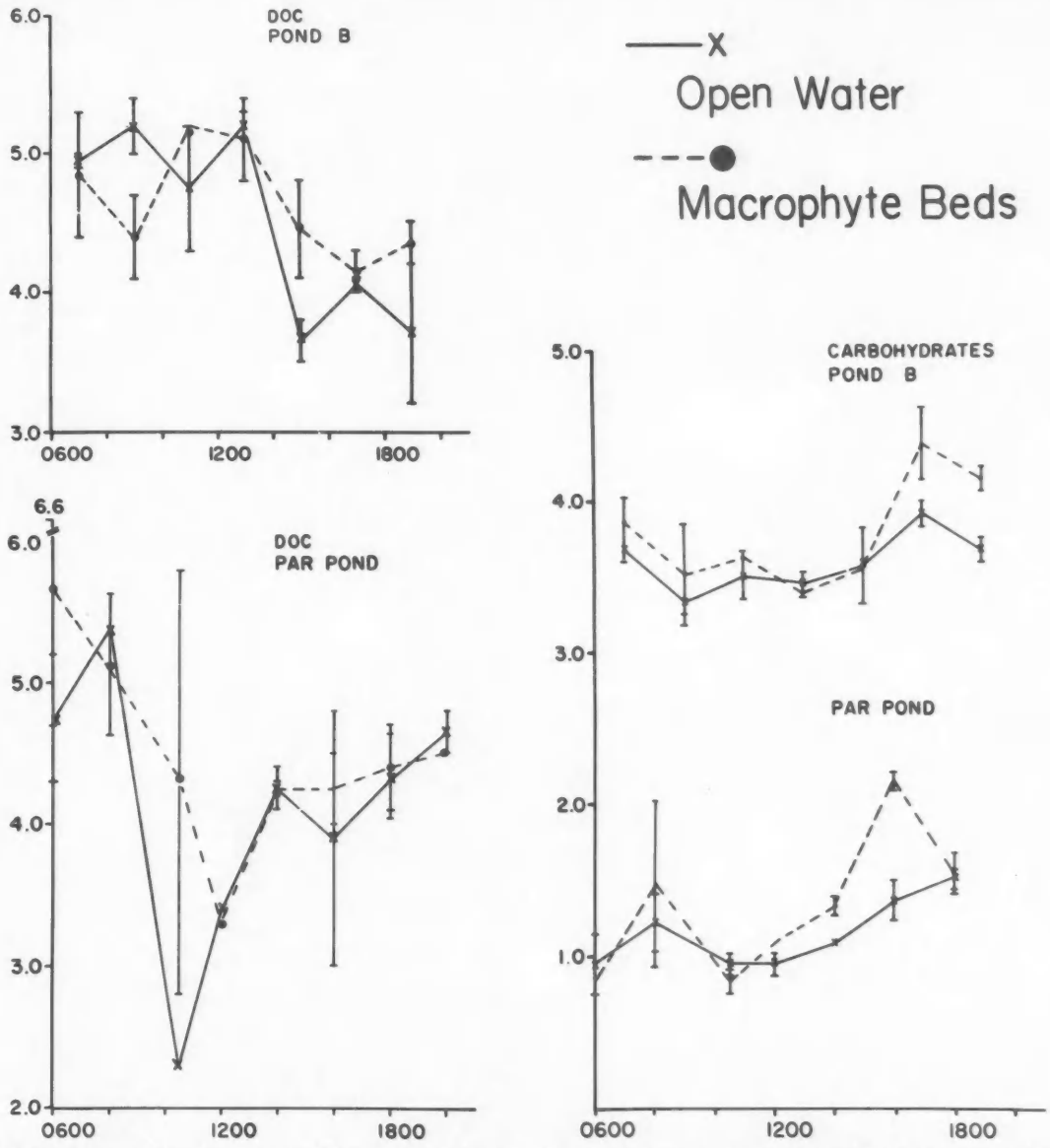

FIG. 3. Diurnal concentrations (mg C·L^{-1}) of DOC and dissolved carbohydrates in open waters and macrophyte beds at Station 3 in Pond B and Par Pond on 11 August 1982.

TABLE 2. Average dissolved carbohydrate concentrations (mg·L^{-1}) in two ponds during 1982–83. Reported error is ±1 SD of the mean for *N* samples.

	Pond B		Par Pond	
	Mean	*N*	Mean	*N*
All values	2.86 ± 1.01	104	1.72 ± 1.24	111
Surface water only	3.14 ± 0.88	78	1.85 ± 1.37	82
Surface open water	2.95 ± 0.81	45	1.76 ± 1.25	45
Surface macrophyte beds	3.41 ± 0.90	34	1.97 ± 1.52	37

of excretion on factors such as the concentration of mono- and divalent cations (Wetzel 1969b). The concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} measured in these ponds (Alberts and Dickson 1985) are relatively low and should place the rate of excretion near the maximum as determined for *N. flexilis*. Since *N. guadalupensis* is a dominant species at least in Par Pond and the rooted macrophyte zones in Pond B and Par Pond comprise approximately 55 and 45% of the reservoir surface areas, respectively (Boyd 1970; Grace 1977), it might be expected that carbohydrate excretion by rooted plants would be seen as a significant increase in the dissolved carbohydrate concentrations of the macrophyte beds relative to the open waters.

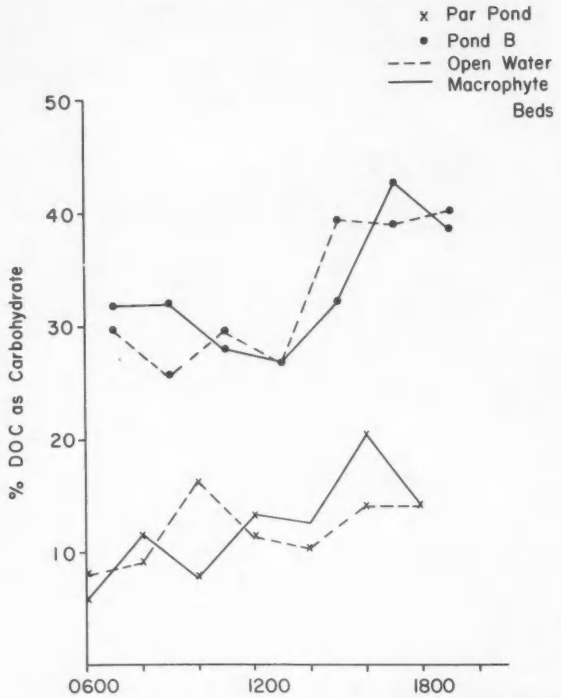

FIG. 4. Diurnal percentage of the DOC that is dissolved carbohydrate in open waters and macrophyte beds at Station 3 in Pond B and Par Pond on 11 August 1982.

We find little evidence that the macrophyte beds have consistently higher concentrations of dissolved carbohydrates; however, there is an indication that bays which have dense macrophyte stands and little exchange with the open waters of the reservoirs do have higher concentrations of carbohydrates in the macrophyte beds (Station 2 in both systems). This weak trend and the elevated carbohydrate concentration in the macrophyte beds relative to the open waters, which occurs in the late afternoon in both systems, are the only indication of a build-up of carbohydrates due to macrophyte excretion.

Several possible explanations could account for the dissolved carbohydrate trends observed in these softwater systems: (1) macrophyte excretion may not occur or may be negligible relative to matter from planktonic organisms, allochthonous inputs, and decomposition; (2) macrophytes excrete dissolved organic matter of which carbohydrates are not an important constituent; (3) macrophytes excrete carbohydrates, but water circulation usually removes this production rapidly to the bulk waters; and (4) macrophyte excretion of carbohydrates may be seasonal (Rogers 1965) and, coupled with low excretion rates for other species more prevalent than *Najas*, may cause the carbohydrate excretion to be lost in the noise of the analyses.

Vertical Carbohydrate Distribution

In contrast with the spatial and temporal trends, the vertical water column distribution of carbohydrates was distinctly different between the two ponds. On all sampling dates, Pond B had concentration minima between 8 and 10 m, while Par Pond had concentration maxima between 5 and 6 m. In Pond B, the minima occurred several metres below the chemocline during stratification but occurred at the point of lowest oxygen concentration during April 1983. During stratification of Par Pond, the depth of the maxima was coincident with the thermocline. However, the highest carbohydrate maximum occurred at the same depth during holomixis.

A variety of depth distributions of carbohydrates have been reported. Münster (1984) also noted considerable variation in the concentrations of dissolved carbohydrates with depth in the Plussee whereas in Lake Kinneret, glucose concentrations varied little with depth from March to October (Cavari and Phelps 1977). Neither of these two studies showed a consistent pattern of increase or decrease in carbohydrate concentrations with depth. However, a concentration increase similar to that observed in Par Pond, i.e. occurring at the depth at which oxygen begins to be depleted from the water column, was reported in Japan (Ochiai and Hanya 1980a).

During the August samplings in both years, oxygen concentrations were zero below the thermocline. Calculations of relative thermal resistance (Wetzel 1975) show that an intense pycnocline was established at the depth of the thermocline, which may have reduced downward transport of material (Alberts et al. 1988). Thus, if the carbohydrates in the hypolimnia were residual from surface waters, they would have been the more resistant sugars such as ribose and arabinose (Ochiai and Hanya 1980b), since decomposition of dissolved carbohydrates in lake waters appears to occur at approximately the same rate in anaerobic and aerobic waters (Ochiai et al. 1980b). Alternatively, the dissolved carbohydrates in the hypolimnion may represent material derived from particles settling through the pycnocline or arising from anaerobic processes in the sediments. The increased dissolved carbohydrate concentrations observed in the deepest waters of Pond B on both occasions and in Par Pond during August 1983 lend support for the latter hypothesis.

The peak in dissolved carbohydrate concentration observed in Par Pond during the period of holomixis is likely the result of biological activity rather than physical processes, as no thermal structure is apparent in the water column at that time. However, other than the fact that the peak depth is coincident with the bottom of the euphotic zone in the reservoir, little evidence is available to support a biological mechanism by which this concentration maximum could be maintained. The depth distribution of primary producers, quantitative data on the distribution of specific carbohydrates, and estimates of the relative sizes of the heterotrophic bacterial pools are required to determine if biological or physical processes are controlling the depth distribution of carbohydrates in this system.

Potential Dynamics of Carbohydrates

The dissolved carbohydrate concentrations in both reservoirs are a substantial portion of the DOC. During the diurnal study, the carbohydrates comprise 6–20% of the DOC in Par Pond, while in Pond B they represent 26–42% of the total DOC (Fig. 4). Although one previous study in a freshwater system has reported 30% of the DOC to be dissolved carbohydrates (Ogura and Gotoh 1974), our values are high relative to the majority of published data, which generally range from 8 to 12% (Thurman 1985).

Natural phytoplankton populations can release up to 70% of the total fixed carbon as extracellular DOC (Rai 1984), while freshwater planktonic bacteria can remove up to 15% of the total DOC per day (Allen 1973). However, the rate of respiration of this material is quite dependent on the structure of the

compound being utilized. If extracellular carbohydrates in this study were being generated from light-induced production, the material being added must have been readily utilizable by heterotrophs or rapidly transported through the water column, since the late afternoon carbohydrate production appears to be lost during the night.

Assuming first-order kinetics and requiring that all dissolved carbohydrate produced during the day be metabolized before the next morning, the diurnal data yield mean rate constants for dissolved carbohydrate decomposition of 0.29 and 1.18·d^{-1} in Pond B and Par Pond, respectively. These values are in agreement with the value of 1.24·d^{-1} calculated during the initial 3 d of decomposition of dissolved carbohydrates from diatoms (Ogura and Gotoh 1974). In laboratory experiments, the total dissolved carbohydrate pool was depleted at a rate of 3.08×10^{-2}·d^{-1} and glucose was depleted at a rate of 8.17×10^{-2}·d^{-1} (Ochiai and Hanya 1980b). Using those rate constants and data from the diurnal study, it would take approximately 6 d to metabolize the daily carbohydrate production in Pond B if it were mixed carbohydrates and approximately 2 d if it were glucose. In Par Pond, mixed carbohydrates would require about 22 d and glucose about 8 d to be metabolized. Thus, it appears that the increases in dissolved carbohydrate concentrations in the afternoon and evening are readily assimilated sugars similar to those excreted by diatoms, and/or late evening and night time physical mixing is sufficient to dilute this daily production into the bulk waters of the ponds.

Conclusions

This study has provided the first data on the distribution and dynamics of total dissolved carbohydrates in two southeastern United States reservoirs. Data presented here indicate that up to 42% of the DOC may exist as carbohydrates and that the microbial/planktonic communities of these systems are more important in controlling dissolved carbohydrate dynamics than the rooted macrophyte communities. The depth distribution of total dissolved carbohydrates shows substantial concentrations of these compounds in the anoxic waters of both systems. These waters have been anoxic for at least 3 mo and the presence of carbohydrates is indicative of a source of material either from the epilimnion or the sediments.

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A Maximum Likelihood Methodology for Estimating Length-at-Maturity with Application to Pacific Cod (*Gadus macrocephalus*) Population Dynamics

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Welch, D. W., and R. P. Foucher. 1988. A maximum likelihood methodology for estimating length-at-maturity with application to Pacific cod (*Gadus macrocephalus*) population dynamics. Can. J. Fish. Aquat. Sci. 45: 333–343.

A maximum likelihood methodology for the analysis of maturity-at-length data is presented, and illustrated with data for female Pacific cod (*Gadus macrocephalus*) from Hecate Strait, British Columbia. Our approach explicitly accounts for the binomial nature of maturity data. Estimated maturity–length relationships show substantial differences for females sampled from different grounds within Hecate Strait, as well as large between-year differences in the maturity–length relationships for individual grounds. However, the maturity–length characteristics for a single ground are stable thoughout the spawning season, and we argue that the observed temporal variability is unlikely to be a sampling artefact. Assuming constant somatic growth rates, these differences correspond to shifts of slightly more than 1 yr in the median age-at-maturity; this shift implies that the instantaneous population growth rate r_m for Pacific cod can increase by up to $0.25 \cdot yr^{-1}$ in years when maturation occurs at smaller body size. This corresponds to an increase of over 50% in the sustainable fishing mortality rate, independent of other factors. Our results suggest that more attention should be focussed on assessing the reproductive dynamics of adult fish.

Les auteurs présentent une méthode de vraisemblance maximale pour l'analyse de données sur la maturité selon la longueur, méthode qu'ils démontrent à l'aide de données sur des femelles de morue du Pacifique (*Gadus macrocephalus*) capturées dans le détroit d'Hécate (Colombie-Britannique). L'approche des auteurs tient explicitement compte de la nature binomiale des données sur la maturité. Les relations déterminées entre la maturité et la longueur révèlent des différences marquées entre les femelles prélevées dans différentes frayères du détroit d'Hécate ainsi que d'importantes différences inter-annuelles pour ce qui est des relations entre la maturité et la longueur dans chaque frayère. Toutefois, les caractéristiques maturité–longueur d'une frayère sont stables pendant toute la saison du frai; les auteurs prétendent donc qu'il est peu probable que la variabilité temporelle observée soit un artefact d'échantillonnage. Si l'on suppose des taux constants de croissance somatique, ces différences correspondent à des décalages d'un peu plus d'un an de l'âge médian à la maturité; ce décalage signifie que le taux instantané de croissance de la population (r_m) chez la morue du Pacifique peut augmenter d'un facteur allant jusqu'à $0{,}25 \cdot an^{-1}$ pendant les années où la maturation a lieu à une plus petite taille corporelle. Ceci correspond à une augmentation de plus de 50 % du taux soutenu de mortalité par pêche, indépendamment d'autres facteurs. Les résultats portent à croire que l'évaluation de la dynamique de la reproduction des poissons adultes devrait faire l'objet d'études plus poussées.

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Changes in rates of sexual maturation for marine fish are frequently reported (e.g. Alm 1959; Beacham 1982a, 1982b, 1983a, 1983b, 1983c, 1983d, 1983e, 1983f, 1983g; Clark et al. 1982; Dragesund et al. 1980; Hislop and Shanks 1980; Hubold 1978; Pinhorn 1969; Powles 1958; Schmitt and Skud 1978; Sinclair et al. 1982; Templeman et al. 1978; de Veen 1976). The consequences of these changes for the regulation of population productivity have been stressed by Cole (1954) and Stearns (1976) and are qualitatively recognized by most workers. However, in most studies, these changes are simply reported as shifts in the median age- or length-at-sexual maturity.

Two points need to be made about these studies. First, most studies are based on a rather qualitative analysis of the data, so uncertainty in the stated estimates of the 50% maturation point are frequently difficult to evaluate. Second, even when the underlying data are sufficiently "good" that changes in maturity are clear-cut, the population consequences are rarely evaluated; for example, their effect on the population growth rate, and thus on sustainable fishing mortality rates, is not quantified.

In this paper we present a general methodology for the study of changes in maturity-at-length or maturity-at-age that takes into explicit account the binomial nature of maturity data. We illustrate the utility of our approach with an analysis of changes in the maturity-at-length scheduie for female Pacific cod (*Gadus macrocephalus*) from Hecate Strait, British Columbia.

Early work demonstrated that female Pacific cod mature at smaller sizes in Hecate Strait than do individuals from more

boreal regions (Thomson 1962). We reexamined these data to establish whether the maturity–length relationship for Hecate Strait cod has remained constant. Our analysis compared the maturity-at-length schedules obtained from research data collected in the early 1960's with more contemporary data obtained from port sampling of commercial fishing vessels. The results indicate that between-year changes in the median length-at-maturity of approximately 10 cm (from 55 to 45 cm) may have occurred. Assuming constant somatic growth rates, this corresponds to a decrease from 3 to 2 yr in the median age-at-maturity and has a large influence on population productivity.

Materials and Methods

Hecate Strait cod are believed to spawn primarily on the White Rocks – Bonilla ground (Statistical Area 5D, 53°40′N, 130°45′W) during January–March (Ketchen 1964), although some spawning occurs in other areas as well. Most commercial landings originate from the White Rocks ground in winter, but occasional catches are also reported from other trawl grounds (Fig. 1). We restricted our primary analysis to females taken from the White Rocks ground during the spawning season, since in most years this was the only source of cod for which detailed information was available. We also examined evidence for geographic variation in the maturity–length relationship within Hecate Strait (currently thought to comprise a single stock), but our analysis was largely restricted to 1980, when replicate maturity samples were available from geographically distinct grounds. The origin of commercial samples was established from interviews conducted with vessel masters as a routine part of the commercial sampling program.

Research cruises to Hecate Strait were made in 1960–61 by the *A. P. Knight*, in 1976 by the *G. B. Reed* (Westrheim 1977), and again in January 1987 by one of us (Foucher) on board a chartered commercial fishing vessel, the F/V *Nucleus* (Foucher et al. 1987). The maturity data for 1960–61 were reported by Thomson (1962), but were graphed as percent mature by 5-cm length groups and included an unknown number of observations based on port sampling of commercial landings. We retrieved the original research data from the archives of the Pacific Biological Station, where length was recorded to the nearest centimetre. We could not find the 1960–61 port sample data included in Thomson's (1962) report, so the 1960–61 maturity-at-length estimates reported below are based solely on research samples.

Port sampling of commercially caught female Pacific cod began in 1973, when fish were classified as immature, mature, ripe, or spent. Beginning in 1976, maturity was classified according to a seven-point maturity scale (Westrheim 1977, table 12). We retrieved the available data from computer files maintained at the Pacific Biological Station and converted the seven-point scale into either immature or mature fish, since the finer scale was irrelevant for our needs.

The first useful port sample we could obtain was for 1975, and no port sample data are available for the years 1976, 1977, 1981, and 1984. All data used in this study, including the 1960–61 research data obtained by the *A. P. Knight*, are archived in Foucher and Welch (1987). For all years, fork length was recorded to the nearest centimetre and sexual maturity was defined from examination of the ovaries. We classified females recorded as maturity states 1 and 2 (ovaries small and translucent or colouring) as immature and states 3–7 (ovaries large and opaque, or spent) as mature.

Analytical Methods

A characteristic feature of maturity or sex ratio data is the binomial distribution of the observations; that is, a fish may be either male or female, or mature or immature, but the individual observations do not take on a continuous range of values. Curiously, we are unaware of any previous research on maturity analysis that takes this important attribute of the data into account.

Leslie et al. (1945) proposed a method for estimating median size at maturity based on a least squares criterion using probit analysis which has since been widely applied. We initially considered this approach, but rejected it because of three undesirable characteristics of the residuals.

First, the largest residuals are usually those with small sample sizes. Ordinary least squares parameter estimates will therefore tend to be strongly influenced by the most unreliable observations. To take a concrete example, consider the influence of a maturity observation made for some length group at which only two fish were sampled, and where the true proportion mature is 0.10. Only three estimates of the proportion mature are possible: 0, 0.5, and 1.0. The probability of observing these fractions are, respectively, 0.81, 0.18, and 0.01, so the observations most at variance with the true value are those least likely to be observed, as expected. However, in a least squares framework the effect of these rare observations tends to dominate the analysis. Here the expected contribution is determined by the squared difference between the true and observed proportions multiplied by the probability of making the observation. In this case, the relative contributions are 0.0081, 0.0298, and 0.0081. Thus the most and least likely observations to occur (0 and 100% mature) will have equal weight in fitting a curve by least squares, while the observation of 0.5 will be expected to dominate the fitting procedure.

Second, the *sign* (direction) of the residual is also important, since the probability density function for binomially distributed variables is asymmetric if the true proportion is not 0.5. Least squares estimation procedures cannot easily take this asymmetry into account, as the above example illustrates.

Third, we found for our data set that the residuals about fitted curves are not normally distributed (the fitted curve is described below). While this observation alone does not preclude the use of least squares for estimation, it does have serious consequences for attempts to determine the reliability of the estimates. Most of our maturity observations tend to be at length groups containing either all mature or all immature individuals, with few observations in the transition interval. The frequency distribution of these residuals was typically very peaked, due to the good fit of the model in the 0 or 100% mature regions, but with long tails due to the residuals with small sample sizes. Attempts to normalize the distribution proved only partly successful.

The use of ordinary least squares to estimate the dependence of maturity on length is therefore questionable, but it is difficult to establish a transformation or an objective weighting procedure that can address these criticisms within the least squares framework. We therefore developed a maximum likelihood procedure for fitting binomially distributed data which explicitly takes these concerns into account. In addition, we present a methodology for examining the uncertainty in the parameter estimates in terms of the *plausibility* that some set of alternative parameter values can reasonably describe the data.

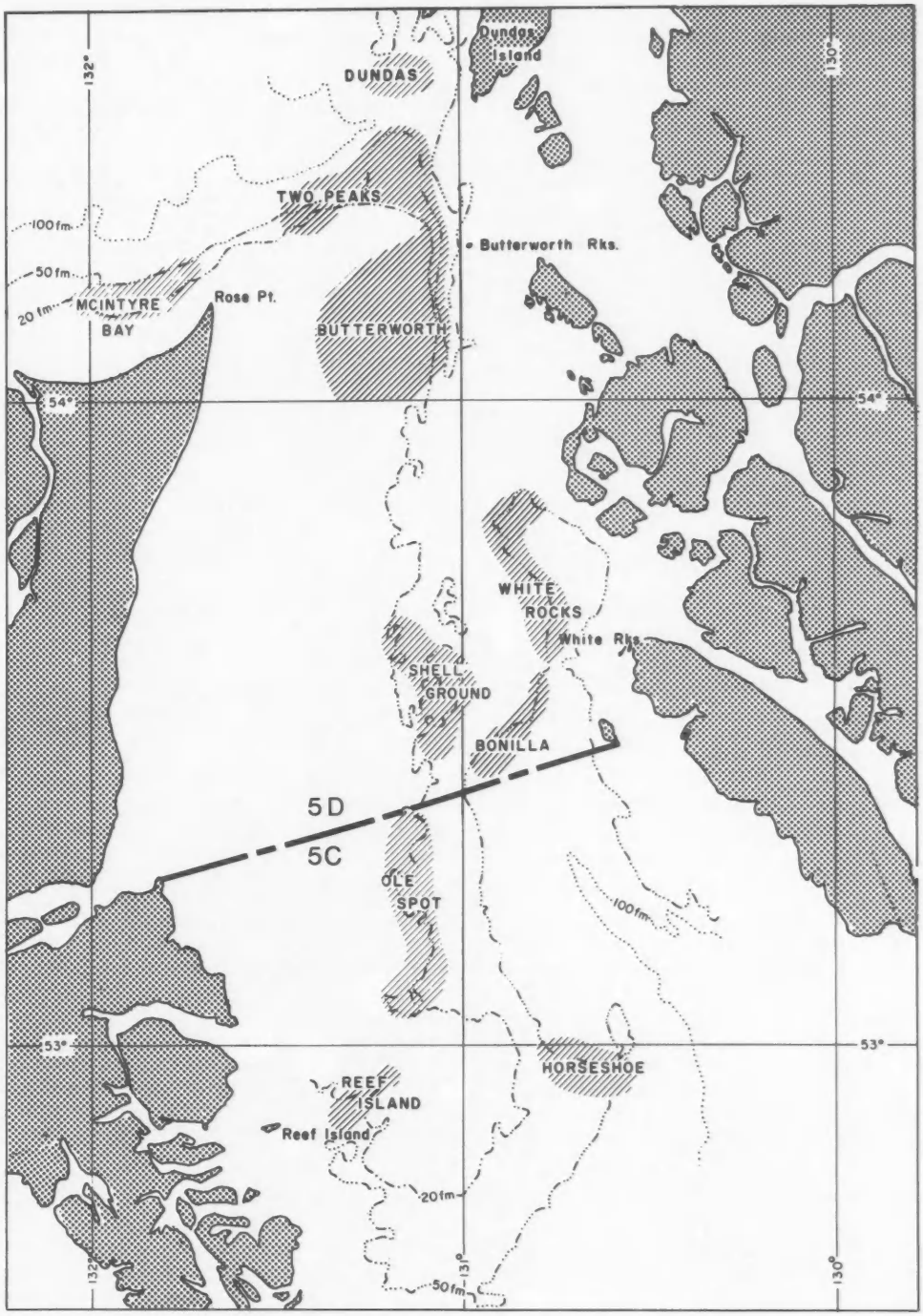

FIG. 1. Hecate Strait, British Columbia, and trawl grounds (1 fm = 1.83 m).

Estimation of Maturity Parameters

Maturity at length x_i, $\phi(x_i)$, is typically observed for a set of M length groups, $i = 1,\ldots,M$. We assume in what follows that these groups correspond to 1-cm length classes and that the error in grouping fish by 1-cm intervals is negligible. Defining r_i as the number of sexually mature individuals observed in the ith length group and N_i as the total number of individuals sam-

pled, then the probability distribution for the fraction mature in each length group, $\phi(x_i) = r_i/N_i$, is binomially distributed and of the form

$$(1)\quad \Pr(\phi(x_i)) = C_{r_i}^{N_i}\ (\phi(x_i))^{r_i}\,(1 - \phi(x_i))^{N_i - r_i}$$

where

$$C_{r_i}^{N_i} = \frac{N_i!}{r_i!\,(N_i - r_i)!}.$$

The likelihood associated with a given set of maturity-at-length observations is then simply the product of the probability distributions at each length group. Therefore, the likelihood over the $i = 1,\ldots,M$ samples is

$$(2)\quad L = \prod_{i=1}^{M} \Pr(\phi(x_i))$$

$$= \prod_{i=1}^{M} C_{r_i}^{N_i}\,(\phi(x_i))^{r_i}\,(1-\phi(x_i))^{N_i - r_i}$$

and the total sample size is $N_{\text{Tot}} = \sum_{i=1}^{M} N_i$.

According to the principle of maximum likelihood, we choose parameter values for the functional form of $\phi(x_i)$ that maximize the likelihood of the function representing the observed sample. This also maximizes the log-likelihood, $\ln(L)$. Thus:

$$(3)\ \ln(L) = \sum_{i=1}^{M} \ln(C_{r_i}^{N_i}) + r_i \ln(\phi(x_i)) + (N_i - r_i)\ln(1-\phi(x_i)).$$

In what follows, we have chosen the two-parameter sigmoid function

$$(4)\quad \phi(x_i) = [1 + (a/x_i)\,e^{-bx_i}]^{-1}$$

as our mathematical model of the maturation process. We choose eq. (4) instead of the more common sigmoid model $\phi(x_i) = [1 + ae^{-bx_i}]^{-1}$ because the former model is zero at zero length, whereas the latter model predicts a maturity rate of $1/(1 + a)$. Thus in the latter model one parameter is used to force the maturity function close to zero at zero length, while in the former model both fitted parameters provide information about what happens once maturation is actually initiated.

Substituting eq. (4) into (3) provides us with an expression for the log-likelihood of the sigmoid model for a given data set:

$$(5)\ \ln(L) = \sum_{i=1}^{M} (N_i - r_i)\,\{\ln(a) - bx_i - \ln(x_i)\} - N_i \ln\left\{1 + \frac{a}{x_i}\,e^{-bx_i}\right\}$$

where we have ignored the contribution of the constant $\Sigma_i\, C_{r_i}^{N_i}$ because its value does not depend on the parameters of the sigmoid curve. Notice that the sample sizes available at each length group enter explicitly into the calculation of the likelihood function (5). Thus the maturity data for length groups with large sample sizes receive more weight in the estimation procedure than do data from length groups with few observations.

Maximum likelihood parameter estimates can be found by minimizing the negative log-likelihood of expression (5). Model parameters reported later were estimated using the simplex search method (Nelder and Mead 1965; O'Neill 1971), using a microcomputer-based Pascal implementation of the code written by Dr. Jon Schnute (Pacific Biological Station, Nanaimo, B.C. V9R 5K6).

The shape of the sigmoid curve is shown in later sections, but we have found it to be an adequate description of the maturation process in Pacific cod. While the two sigmoid parameters (a, b) have no intrinsic biological meaning, they can easily be converted to more familiar values. In this study, we have chosen to report the characteristics of the sigmoid maturity model chiefly in terms of the length at 50% sexual maturity, $X_{0.5}$, and the arithmetic slope at this point, $\phi'(X_{0.5})$, rather than in terms of the model parameters directly estimated (a, b). The maximum likelihood estimates of the former values may be derived from the maximum likelihood estimates $\hat{a}_*$ and $\hat{b}_*$ as

$$(6)\quad \ln(\hat{a}_*) - \ln(X_{0.5}) - \hat{b}_* X_{0.5} = 0$$

and

$$(7)\quad \phi'(X_{0.5}) = \frac{\hat{b}_* + 1/X_{0.5}}{4}.$$

Equation (6) was solved numerically for $X_{0.5}$ by Newton–Raphson iteration.

Plausibility Regions

One method of assessing the uncertainty in maximum likelihood parameter estimates of the sigmoid model is in terms of 95% confidence intervals. As we have already noted, the residuals tend to be nonnormal. More seriously, relatively few of the observations on Pacific cod are in the 45–60 cm range over which the majority of females mature, and residuals within this interval tend to be larger than residuals outside this length interval. Excluding the data outside this range (where the model fits well) in an attempt to improve the normality of the residuals drastically reduced sample sizes.

An alternative approach is possible however, in terms of the *plausibility*, or relative likelihood, that different parameter estimates can reasonably represent the data.

Maximum likelihood parameters are defined as those parameter estimates most likely to represent the data. Associated with these estimates ($\hat{a}_*$, $\hat{b}_*$) is a specific value for the likelihood function (5), L_*. The *plausibility* with which an alternative set of parameter values represent the data can then be defined in terms of the likelihood of these values, L_{alt}, relative to the maximum likelihood, L_*. If the ratio L_{alt}/L_* is near unity the alternative parameter estimates can be thought of as representing the data with nearly equal plausibility.

Since we typically estimate the log-likelihood rather than the likelihood, it is simpler to work in terms of differences in the log-likelihood. Plausible alternative estimates to the maximum likelihood estimates are usually taken as a difference of 2 log-likelihood units or less (Edwards 1984). This corresponds to accepting as plausible all estimates with a likelihood relative to the maximum of at least 13.5% ($e^{-2.0}$). For our purposes, we chose to be somewhat more conservative, accepting as plausible all parameter values within a range of 3 log units of the maximum likelihood. This corresponds to rejecting as implausible all parameter estimates with less than 5% ($e^{-3.0}$) relative likelihood. This choice was made prior to carrying out the statistical analysis reported below.

TABLE 1. Summary of 1987 research (R) and port (P) sample maximum likelihood estimates of maturity-at-length, White Rocks trawl ground. a. and b. are the maximum likelihood estimates of the sigmoid parameters, $X_{0.5}$ and $\phi'(X_{0.5})$ are the derived statistics median length-at-maturity and slope, $-\ln(L)$ is the value of the likelihood function at the maximum, and N_{Tot} is the total sample size.

Sample number	Date (d/mo/yr)	$\hat{a}$.	$\hat{b}$.	$\hat{X}^*_{0.5}$	$\hat{\phi}^{*\prime}(X_{0.5})$	$-\ln(L)$	N_{Tot}
		(a) Individual port sample statistics					
P1	14/1/87	7.92×10^{9}	0.339	55.4	0.089	41.9	194
P2	15/1/87	4.30×10^{11}	0.417	54.6	0.109	36.4	223
P3	19/1/87	7.15×10^{12}	0.488	52.6	0.127	12.7	107
P4	22/1/87	4.90×10^{8}	0.285	56.1	0.076	50.4	210
P5	28/1/87	2.26×10^{8}	0.279	54.5	0.074	31.3	140
P6	05/2/87	3.08×10^{10}	0.355	56.7	0.093	38.3	210
P7	19/2/87	9.11×10^{11}	0.427	55.1	0.111	31.0	262
P8	20/2/87	8.98×10^{8}	0.301	55.2	0.080	33.8	140
P9	26/2/87	4.56×10^{10}	0.378	54.3	0.099	42.0	238
P10	19/3/87	4.72×10^{10}	0.357	57.5	0.094	60.4	232
		(b) Research and aggregate port sample statistics					
R	10/1/87	2.57×10^{10}	0.371	53.9	0.097	122.3	666
P	(All samples)	2.31×10^{10}	0.358	55.5	0.094	391.0	1956

Plausibility regions were found by searching out from the maximum likelihood estimates along a set of lines at fixed angles. In this study, we chose to represent the perimeter of the plausibility regions as a set of 40 points, each 3 log-likelihood units smaller than the maximum likelihood estimates. The likelihood surfaces were also checked visually to ensure that they were well behaved in the vicinity of the maxima.

An important large sample connection between confidence and plausibility regions should be noted. A 95% confidence region indicates that range of parameter values containing the *true* parameter values with 95% probability, given that H_0 is true. In contrast, the 5% plausibility region indicates that region within which any *individual* parameter estimate has at least 5% of the maximum likelihood of representing the data. These two measures of uncertainty therefore have differing interpretations; however, for large samples the deviance of binomially distributed variables approximates a chi-squared distribution with 1 degree of freedom (McCullagh and Nelder 1983, p. 231), so a 3 log-likelihood difference is asymptotically equivalent to a 95% confidence region when fitting two parameters. We deemphasize this connection, however, first because of the small sample sizes available and second because we feel the concept of plausibility has important merits of its own; see Edwards (1984) for further discussion.

Results

Comparison of Commercial and Research Samples

Annual estimates of the maturity–length schedule presented in a later section of this paper indicate that large changes in the median length-at-maturity of female Pacific cod have occurred with time. However, the greatest differences in median length-at-maturity were observed between port samples collected during 1975–86 and research samples obtained in 1960–61 and 1976. The estimated median length-at-maturity was consistently greater in those years when maturity data were collected by research vessels. As no port samples were taken in 1976, the sampling periods do not coincide.

These differences raise questions concerning the comparability of maturity data derived from the two sampling protocols. We shall first analyze the results of a test designed to establish whether the methods of data collection differ systematically, in order to establish the reliability of the data. This should also help to illustrate the likelihood analysis of the between-ground and between-year variation we subsequently present.

Pacific cod become commercially acceptable to processors at about 45–50 cm total length, the exact length depending on market conditions (Pacific Biological Station, unpubl. data). Culling of undersized cod occurs at sea prior to landing. One possible explanation for the smaller median length-at-maturity estimated from port sample data is that relatively fewer mature cod are discarded from the catch because they appear fatter (and therefore larger) at any given length than do immature females.

The 1987 spawning season provided an opportunity to test this hypothesis. All Pacific cod caught during a research cruise to the White Rocks spawning ground in January 1987 were measured and sexed. The research data indicated that almost all females were immature in the 40–50 cm length range and that maturation occurred over the 50–60 cm length range. This result was nearly identical with the data from research surveys taken in 1960–61 and 1976 and very different from most of the data obtained from port samples in 1975–85.

We therefore requested that a special effort be made by port samplers to obtain as many Pacific cod maturity samples as possible from the White Rocks spawning ground. To improve the definition of the maturation process in the 50–60 cm interval, we also requested that as much additional maturity–length data for females in the 50–60 cm range be collected as time permitted, after regular sampling was completed.

Summary statistics for these data sets are reported in Table 1a, and the raw data are available in Foucher and Welch (1987). Maximum likelihood parameter estimates for the sigmoid maturation model were calculated for each data set by minimizing the negative log-likelihood (5). Five percent plausibility regions about these maximum likelihood estimates were then calculated as described above.

The results show that the plausibility regions about the different point estimates generally overlap, regardless of sampling protocol (Fig. 2a). Since it is therefore plausible that the estimated maturity parameters for any one port sample are representative of the other samples, a second test for systematic differences was made by aggregating all port samples and reestimating the maturity parameters and comparing these parameters with the research sample.

The results of this comparison indicate that there is a very close correspondence between the likelihood estimates derived from the two protocols, and that it is therefore plausible to assume that both research and commercial samples are equally representative of the data for 1987 (Fig. 2b; Table 1b). In fact, the estimated median length-at-maturity for the aggregated commercial data in 1987 is slightly greater than for the research sample estimate, the opposite of the anticipated effect. We therefore tentatively conclude that the between-year differences in maturity schedules described below are unlikely to be an artefact of variation in sampling methodology over time.

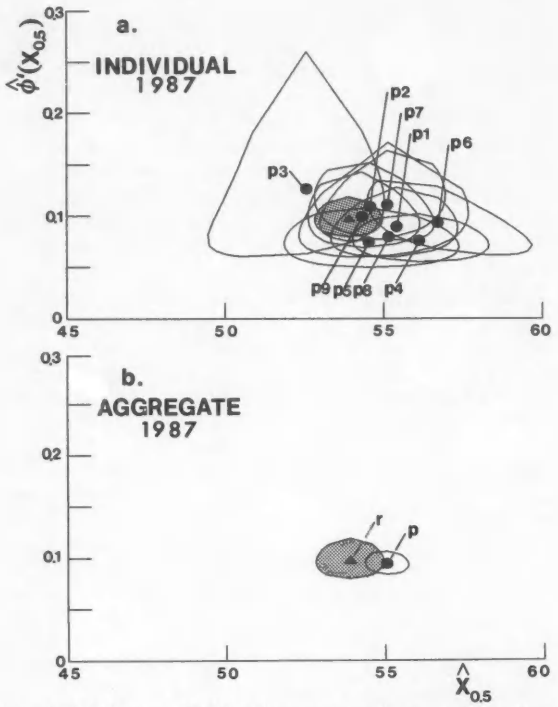

FIG. 2. (a) Maximum likelihood parameter estimates and associated 5% plausibility regions of median length-at-maturity, $X_{0.5}$, and slope, $\phi'(X_{0.5})$, calculated for the 1987 research data (triangle) and commercial samples (circles). The plausibility region for the research sample is shaded. (b) Maximum likelihood parameter estimates and associated 5% plausibility regions calculated from the aggregate 1987 commercial data (circle) and compared with the 1987 research sample (triangle).

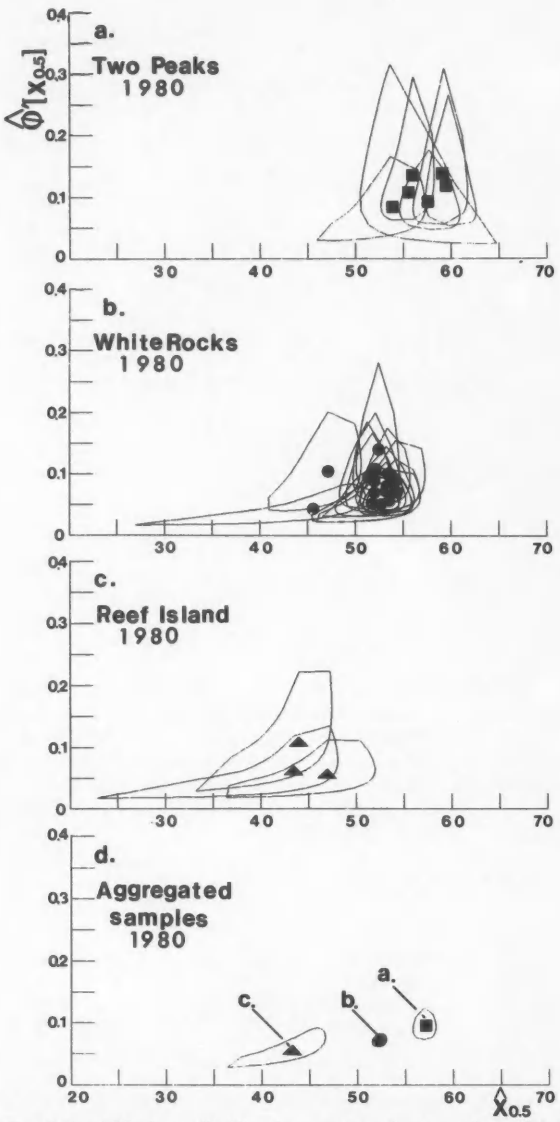

FIG. 3. (a–c) Maximum likelihood parameter estimates and associated 5% plausibility regions of median length-at-maturity, $X_{0.5}$, and slope, $\phi'(X_{0.5})$, calculated for individual port samples taken from the Two Peaks – Butterworth (squares), White Rocks (circles), and Reef Island grounds (triangles) in 1980. (d) Maximum likelihood parameter estimates and associated 5% plausibility regions calculated from the aggregated 1980 commercial data for each ground.

Between-Ground Variability in Maturity-at-Length

Between-ground differences in the geographic distribution of sexually mature and immature females have previously been reported for Atlantic cod (*Gadus morhua*) (Brander 1975) and for Pacific halibut (*Hippoglossus stenolepis*) (Skud 1977). Evidence for geographic heterogeneity has not previously been examined for the Hecate Strait stock of Pacific cod, but it is known that the maturity–length characteristics of Hecate Strait cod differ from those reported for other regions of the North Pacific (Thomson 1962; Teshima 1984) and from those reported for the west coast of Vancouver Island (Westrheim 1977).

We examined this possibility by comparing the fitted maturity–length relationships for three grounds within Hecate Strait in 1980, the year for which the greatest number of port samples are available. These grounds (Two Peaks – Butterworth, White Rocks, and Reef Island) span the length of Hecate Strait (Fig. 1). Port samples of Pacific cod from other trawl grounds are uncommon because fishing is concentrated on the White Rocks ground during the spawning season. The maximum likelihood estimates (Fig. 3a–3c; Table 2) give little evidence that port samples from a given ground differ, since individual plausibility regions generally overlap the maximum likelihood estimates for other samples. Accordingly, we aggregated the individual samples and reestimated the maturity parameters for each of the three grounds. The results (Fig. 3d; Table 2b) indicate that the maturity–length relationship is stable during the spawning season for each ground examined and that the between-ground differences in the maturity characteristics of Hecate Strait cod are much larger than the variation observed in the individual port samples.

Between-Year Variability in Research Samples

Examination of the aggregated maturity data available for the White Rocks ground during the period 1960–87 reveals clear

TABLE 2. Summary of 1980 maximum likelihood maturity-at-length estimates for Two Peaks – Butterworth (TP), White Rocks (WR), and Reef Island (RI) trawl grounds. Column headings as in Table 1.

Sample number	Date (d/mo/yr)	$\hat{a}$.	$\hat{b}$.	$\hat{X}^*_{0.5}$	$\hat{\phi}^{*\prime}(X_{0.5})$	$-\ln(L)$	N_{Tot}
		(a) Individual port samples					
TP1	11/02/80	7.60×10^{13}	0.466	59.8	0.121	14.8	59
TP2	12/02/80	4.53×10^{10}	0.355	57.7	0.093	16.0	63
TP3	20/02/80	1.71×10^{9}	0.322	53.7	0.085	12.4	50
TP4	22/02/80	3.53×10^{14}	0.526	56.1	0.136	12.1	61
TP5	10/03/80	3.54×10^{15}	0.535	59.3	0.138	10.5	51
TP6	31/03/80	3.44×10^{11}	0.407	55.4	0.106	20.3	107
WR1	08/01/80	1.97×10^{9}	0.337	51.8	0.089	23.7	53
WR2	10/01/80	1.36×10^{11}	0.416	52.1	0.109	18.4	54
WR3	10/01/80	3.52×10^{6}	0.211	52.7	0.057	107.1	194
WR4	15/01/80	4.00×10^{7}	0.254	53.2	0.068	38.9	81
WR5	24/01/80	6.85×10^{9}	0.365	51.3	0.096	20.2	55
WR6	28/01/80	7.28×10^{8}	0.309	53.3	0.082	27.4	62
WR7	29/01/80	2.07×10^{14}	0.554	52.4	0.143	13.5	60
WR8	13/02/80	4.40×10^{6}	0.213	53.2	0.058	24.3	57
WR9	18/02/80	2.23×10^{7}	0.249	52.0	0.067	25.2	62
WR10	19/02/80	3.60×10^{9}	0.385	47.2	0.102	9.5	60
WR11	25/02/80	4.45×10^{4}	0.151	45.5	0.043	30.4	96
WR12	03/03/80	2.27×10^{10}	0.372	53.4	0.098	21.9	66
WR13	20/03/80	2.07×10^{9}	0.322	54.2	0.085	16.7	58
WR14	25/03/80	1.51×10^{6}	0.198	52.0	0.054	25.1	58
WR15	31/03/80	2.21×10^{8}	0.281	54.2	0.075	33.5	97
RI1	14/01/80	7.03×10^{5}	0.205	47.0	0.056	14.0	49
RI2	21/01/80	7.08×10^{5}	0.223	43.4	0.062	14.1	55
RI3[a]	29/01/80	1.10×10^{-4}	0.550	—	—	0.00	55
RI4	25/02/80	4.96×10^{9}	0.421	44.0	0.111	8.67	74
		(b) Aggregate port samples, by ground, January–March 1980					
TP	1980	4.82×10^{10}	0.361	57.0	0.095	96.8	391
WR	1980	6.32×10^{7}	0.268	52.3	0.072	460.7	1113
RI	1980	1.97×10^{5}	0.196	43.1	0.055	55.2	233

[a]Sample RI3 consisted entirely of mature females. This sample was added to the other Reef Island port samples to form the aggregate sample.

evidence that maturity-at-length has been highly variable during this period (Fig. 4). Note that in those years when substantial numbers of fish were measured, plausibility regions tend to be very tight. In contrast, plausibility regions are broad when few or no small females are present in the maturity samples (cf. 1975, 1978, and 1985), and the region within which different maturity–length relationships can be interpreted as plausibly representing the data is accordingly large.

Maximum likelihood estimates of the derived parameters $\hat{X}^*_{0.5}$ and $\hat{\phi}'(X_{0.5})$ are shown in Fig. 5 along with their associated 5% plausibility regions. These support regions include the maximum likelihood estimates for other years in some cases, but plausibility regions for adjacent years of data overlap in only one instance. Thus, parameter estimates for one year generally have little likelihood of being representative of data collected in other years.

This point is clarified in Fig. 6, where a large drop in median length-at-maturity is evident between 1960–61 and 1975, when no data were collected, followed by erratic between-year fluctuations. The median length-at-maturity estimates for recent (1986–87) samples are, however, very similar to the pattern observed for the data from the early 1960's. In contrast, there is little evidence that the slope at the 50% maturity point has varied systematically with time (Fig. 6b), so most of the observed changes seem to involve a shift in the position of the maturity ogive with respect to length, rather than a change in the shape of the curve.

Maturational Influences on the Population Growth Rate

The maximum likelihood estimates of the annual maturity-at-length relationship are summarized in Table 3 for each year available. To quantify the influence of these changes in maturity-at-length on population productivity we examined the effect of the fitted maturity schedules (Table 3) on the instantaneous population growth rate, r_m.

The characteristic equation for density-independent age-structured populations is (Charlesworth 1980)

$$\sum_t k(t)\, e^{-r_m t} = 1$$

where

$$k(t) = \phi(t) f(t) l(t),$$

so

$$(8)\quad \sum_t \phi(t) f(t)\, e^{-(r_m t + \sum_{i=0}^{t-1} Z_i)} = 1.$$

Here, $\phi(t)$, $f(t)$, and $l(t)$ are, respectively, the fraction mature at age t, the fertility (as eggs) of t-yr-old sexually mature individuals, and the survivorship from fertilization to age t for these eggs. Z_i is the instantaneous mortality from age i to $i + 1$, so $l(t)$ can be replaced in eq. (8) by $\exp(-\sum_{i=0}^{t-1} Z_i)$. We assume female demographic dominance so that only the vital rates of the females need be considered.

To examine the influence of changes in the maturity schedule on population productivity, it is necessary to define the relationship between the various length-specific vital rates and their corresponding values-at-age. Early attempts to assess somatic growth rates in Pacific cod were hampered by difficulties in ageing individuals (Ketchen 1964), so we have no information as to whether somatic growth rates have historically exhibited variability similar to that observed in rates of maturation.

To establish a standard for converting length schedules into age schedules, we have used length-at-age data calculated from first quarter sampling, conducted primarily on the spawning grounds, to define the parameters of a von Bertalanffy length-at-age curve (Table 4). Fertility-at-length parameters are available for the Hecate Strait Pacific cod (Thomson 1962) and are similar to those for boreal populations. These values have been used along with VPA-based estimates of mortality-at-age to define age-specific maturity, fertility, and survivorship schedules (Table 4).

It is possible to compare the *relative* population growth rates r_m achieved under different maturity schedules by converting the length-specific fertility and maturity schedules into age-specific schedules. Since a direct estimate of total mortality experienced in the 2 yr prior to recruitment (i.e. $Z_0 + Z_1$) is unavailable, we have chosen that value necessary to reduce the population growth rate to zero with the estimated 1960 maturity schedule ($Z_{0+1} = 11.66 \cdot \text{yr}^{-1}$). It may be seen by inspection of eq. (8) that r_m varies in the opposite direction to changes in the value of Z_{0+1} when the other parameters are held constant. Thus,

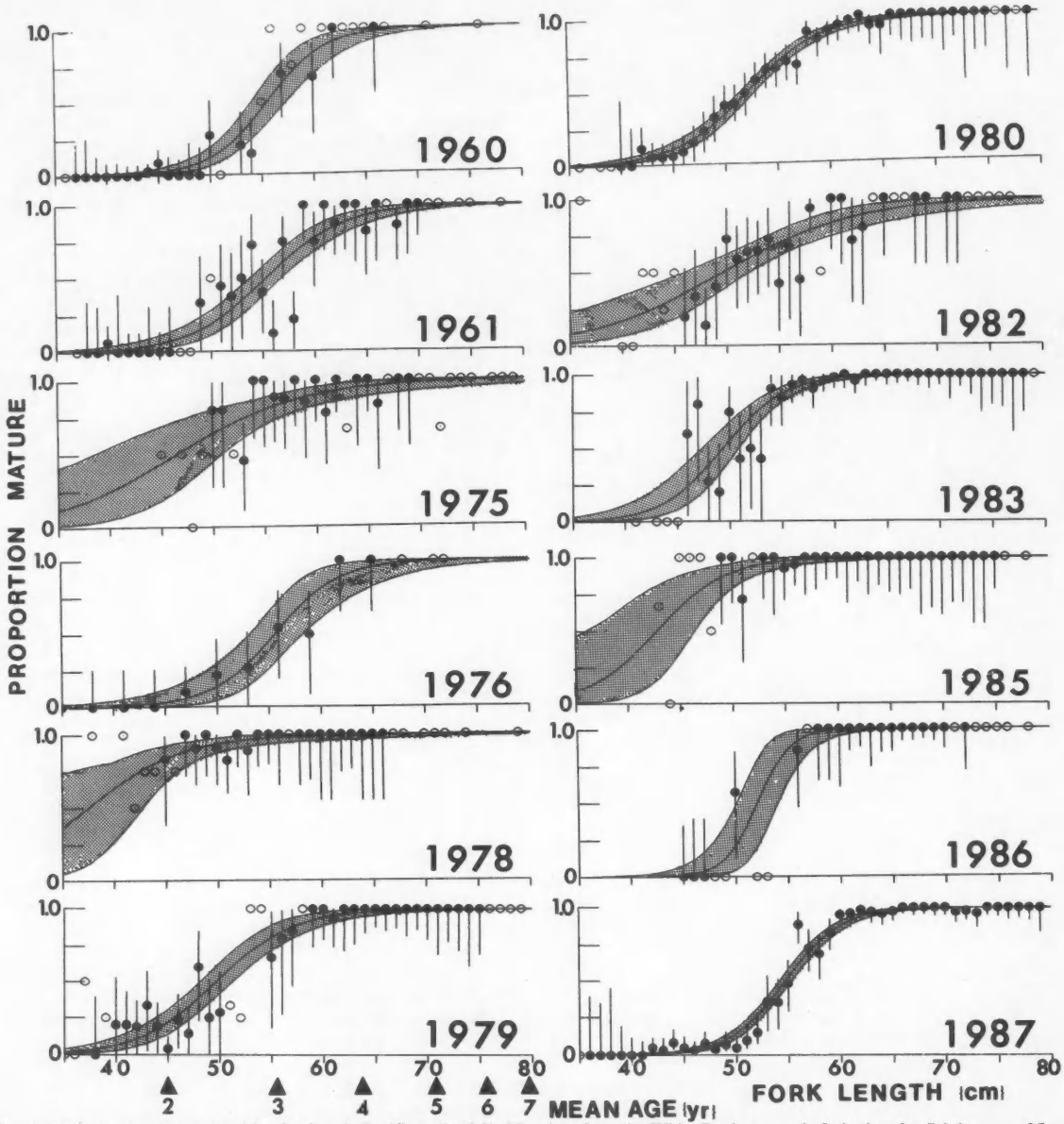

FIG. 4. Maturity–length relationships for female Pacific cod, 1960–87, taken from the White Rocks ground. Only data for fish between 35 and 80 cm fork length are plotted. Closed circles: maturity-at-length samples based on four or more observations; open circles: samples based on three or less observations. Shaded regions indicate the 5% plausibility regions for the fitted curves, and vertical bars indicate the 95% confidence regions on those maturity-at-length observations that are based on four or more observations. Upper and lower bounds on confidence intervals are based on direct calculation of the limits containing 2.5% of the probability density in each tail of the binomial distribution. For sample sizes of more than 50 individuals per length group, a normal approximation to the binomial confidence limits was used (Pollard 1977, p.214–215). Mean lengths-at-age are indicated at the bottom of the figure.

our comparisons of the population growth rate over time are relative to the 1960 population and cannot be used to infer what the *absolute* value for the intrinsic rate of population increase was in any given year, nor do they consider the influence on r_m of variation in other life history parameters. Note also that the instantaneous fishing mortality rate F is defined by $Z = F + M$, so that variations in fishing mortality must be of the same size but opposite sign to fluctuations in r_m if a fixed population size is to be maintained.

r_m was calculated from eq. (8) using Newton's method for finding the roots of a polynomial (Atkinson and Harley 1983, p.67 ff.). A substantial increase in population productivity relative to the 1960 standard is indicated for most of the recent

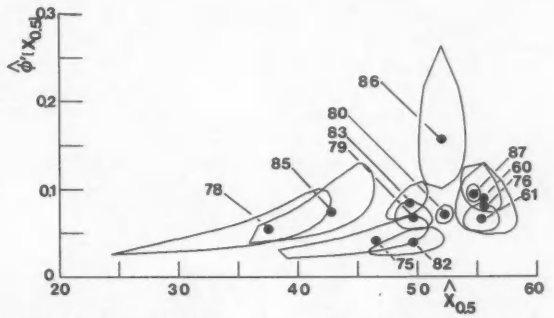

FIG. 5. Maximum likelihood estimates of median length-at-maturity, $\hat{X}_{0.5}$, and slope at 50% maturity, $\hat{\phi}'(X_{0.5})$, for 1960–87. The 5% plausibility regions for the data are also indicated.

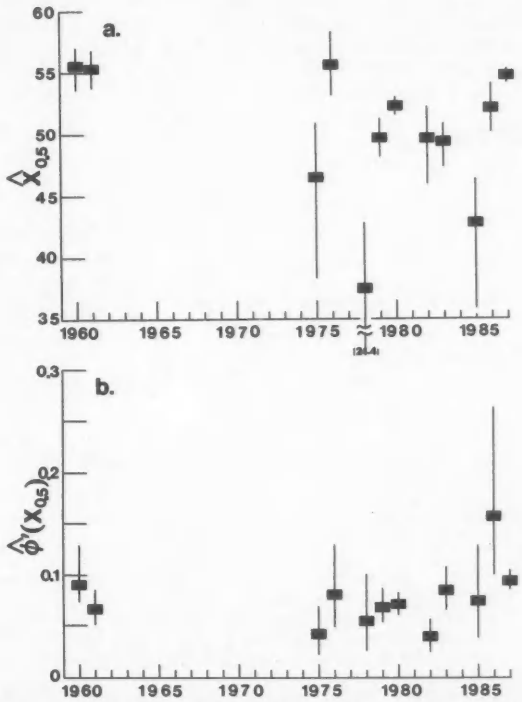

FIG. 6. (a) Variations in 50% length-at-maturity over time. (b) Variations in slope-at-50% maturity over time. The maximum likelihood estimate and 5% plausibility interval are indicated for both graphs.

data (Fig. 7), with instantaneous population growth rates increasing by up to $\Delta r_m = 0.25 \cdot yr^{-1}$. This corresponds to a potential ability to replace up to an *additional* 28% of population biomass in years of rapid maturation (assuming other population parameters remain constant), or the need to vary fishing mortality rates by nearly 60% from their historic mean ($F = 0.44 \cdot yr^{-1}$) if the effect of these changes was to be counteracted.

Discussion

We previously noted several weaknesses in the least squares analysis of maturity data and proposed the use of a maximum likelihood method instead. This approach explicitly takes the binomial nature of such data into consideration. Our results indicate that substantial differences exist in the maturity–length schedules of female Pacific cod sampled from different areas

TABLE 3. Summary of 1960–87 research (R) and port (P) sample maximum likelihood estimates of maturity-at-length. Column headings as in Table 1.

Year	Sample type	$\hat{a}_*$	$\hat{b}_*$	$\hat{X}^*_{0.5}$	$\hat{\phi}^{*\prime}(X_{0.5})$	$-\ln(L)$	N_{Tot}
1960	R	1.06×10^{10}	0.343	55.6	0.090	70.0	436
1961	R	5.25×10^{7}	0.248	55.4	0.067	121.1	357
1975	P	4.22×10^{4}	0.146	46.5	0.042	57.2	178
1976	R	1.36×10^{9}	0.306	55.6	0.081	30.7	115
1978	P	5.19×10^{4}	0.193	37.5	0.055	35.9	216
1979	P	1.43×10^{7}	0.253	49.7	0.068	111.2	452
1980	P	6.32×10^{7}	0.268	52.3	0.072	460.7	1113
1982	P	4.62×10^{4}	0.138	49.7	0.039	112.6	222
1983	P	3.74×10^{8}	0.321	49.4	0.085	109.3	858
1985	P	5.94×10^{6}	0.277	42.8	0.075	26.7	297
1986	P	3.32×10^{15}	0.610	52.1	0.157	14.2	348
1987	P+R[a]	2.43×10^{10}	0.361	55.1	0.095	518.5	2622

[a]Combined research and port sample data reported in Table 1b.

TABLE 4. Data used to calculate age-specific vital rates for Pacific cod.

von Bertalanffy length-at-age parameters[a]

$L_\infty = 97\text{cm}, \qquad K = 0.25 \cdot yr^{-1}, \qquad t_0 = -0.4\ yr$

Fertility-at-length (Thomson 1962)

$f(x) = 0.39\ x^{3.65}$

Mean instantaneous mortality-at-age

(Westrheim and Foucher 1985, table 3.5.8, p. 111)

Age (t)	$0+1$[b]	2	3	4–11
$\hat{Z}(t)$	11.66	0.75	1.0	1.2

[a]von Bertalanffy parameters based on estimated mean length at ages 1–10 yr of 26.0, 45.1, 55.6, 63.8, 70.5, 75.7, 79.8, 83.2, 86.6, and 91.7 cm.

[b]Total prerecruitment mortality necessary to yield zero population growth using the estimated 1960 maturity-at-length schedule.

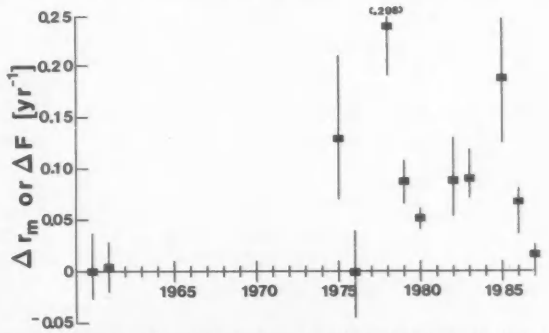

FIG. 7. Influence of variation in the maturation schedule on the instantaneous population growth rate r_m and the sustainable instantaneous fishing mortality rate F, relative to the 1960 standard. Vertical bars indicate the rate of plausible alternative estimates for r_m in different years.

within Hecate Strait (currently thought to be a single stock), as well as large between-year variability in these maturity characteristics. This variability has potentially important consequences for the calculation of population productivity. We are less certain, however, as to the causes of these changes than we are in documenting their occurrence.

The geographic differences in estimated maturity–length schedules clearly illustrate the need for careful averaging when obtaining vital rate estimates to be used in further population analysis; depending on year and/or location, estimates of the sustainable fishing mortality rate for Pacific cod can vary by more than 50%. The maturity characteristics of Pacific cod from a given area do appear to be stable during the spawning season, since individual port samples show high likelihood of being drawn from the same population and little evidence of any temporal trend. If the between-ground differences we observe are due to a differential migration of sexually mature females (e.g. Brander 1975; Skud 1977), then this migration must have been largely completed prior to sampling. Alternatively, the differences could indicate either a population substructure or a geographic variation in somatic growth rates which we are unaware of. These possibilities require further study.

An intense fishery should discriminate against late maturation and select for fast growing early maturing genotypes (Alm 1959). The sharp drop in median length- and age-at-maturity seen in many exploited fish populations provides qualitative support for this hypothesis (see citations in the introduction). However, the temporal changes in maturity for Pacific cod from the White Rocks ground are inconsistent with this mechanism. Median length-at-maturity dropped from 55 cm in 1960–61 to 45 cm in the late 1970's, but then increased again. Our analysis therefore provides little evidence that the remarkable phenotypic plasticity observed is expressed at the genetic level. Further evidence that factors other than genotypic change drive the shifts in length-at-maturity comes from the large changes in median length-at-maturity on short time scales. For example, median length-at-maturity differed by nearly 10 cm between the 1975 and 1976 spawning seasons, and similarly between the 1985 and 1986 seasons. These changes are almost as great as the maximum observed difference during the 27-yr period for which we have data.

Changes in maturity seem to be largely expressed as shifts in the maturity schedule along the length axis, rather than as changes in the shape of the curve once maturation is initiated. If one assumes constant somatic growth rates, the observed changes in maturity correspond to a drop in the median age-at-maturity from 3 to 2 yr and then an increase back up to 3 yr by 1987. Such changes have significant population consequences (Cole 1954). In our study, a 1-yr decline in the median age-at-maturity corresponds to the ability to remove an *additional* 28% of the population each year without changing the standing stock available in the next year, because of the increase in the population growth rate r_m that ensues as maturation occurs at smaller body sizes. These changes are therefore remarkably large. However, ageing of Pacific cod is quite difficult, so we are unable to evaluate the possibility that the observed changes in length-at-maturity are a response to changes in somatic growth rates.

Since body size is an important determinant of both maturity and fecundity, variable somatic growth rates could have an additional effect on population productivity. This is particularly true if somatic growth rates increased in concert with declines in the median length-at-maturity. Pacific cod in Hecate Strait are near the southern limit to their range (Ketchen 1964) and mature at smaller body size and have higher somatic growth rates than populations in more boreal regions (Thomson 1962; Foucher et al. 1984). It is therefore possible that the observed shifts in maturity represent a strategy for allocating energy between somatic growth and reproduction in a seasonal environment, since successful reproduction is limited to a short interval during the year. This possibility needs further study.

Acknowledgements

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Notes

Influence of Seasonal River Discharge on Survival of Juvenile Atlantic Salmon, *Salmo Salar*

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Gibson, R. J., and R. A. Myers. 1988. Influence of seasonal river discharge on survival of juvenile Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 45: 344–348.

The effects of winter and summer river discharge on the survival of eggs and underyearlings of Atlantic salmon (*Salmo salar*) were examined in six rivers of Newfoundland and New Burnswick where stock–recruitment relationships were available. There were positive relationships between either winter discharge or temperature and survival of underyearling salmon for five rivers. For all rivers combined, survival and winter discharge were related ($P < 0.05$). The hypothesis that summer discharge was unrelated to survival could not be rejected. However, there was evidence that summer discharge enhances survival in the Miramichi and Northwest Miramichi rivers of New Brunswick.

Les auteurs ont étudié l'incidence du débit fluvial en hiver et en été sur la survie des oeufs et des tacons O+ de saumon de l'Atlantique (*Salmo salar*) peuplant six rivières de Terre-Neuve et du Nouveau-Brunswick pour lesquelles on disposait de relations sur le recrutement du stock. Ils ont noté des relations positives entre, d'une part, le débit hivernal et la température et, d'autre part, la survie des tacons O+ fréquentant cinq rivières. Ils ont aussi relevé une relation ($P < 0,05$) entre la survie et le débit hivernal dans toutes les rivières regroupées. L'hypothèse selon laquelle le débit estival n'est pas lié à la survie n'a pu être rejetée. Toutefois, certains faits prouvent que le débit estival améliore la survie dans les rivières Miramichi et Northwest Miramichi (Nouveau-Brunswick).

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The production of salmonids in rivers is dependent on a number of factors (McFadden 1969), including stability of river discharge (Binns and Eiserman 1979). In addition to regulating prey abundance (Hynes 1970), discharge has direct effects on salmonid production. Wickett (1958) showed that stream discharge had a positive relationship with survival of pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) when spawners were migrating upstream and during early incubation of the eggs, but a negative relationship in the winter, when floods could displace gravel and eggs or alevins. Similarly, Nickelson et al. (1986) suggested that spring freshets reduced survival of early emerging coho salmon (*O. kisutch*), and Cramer et al. (1985) found a negative relationship between survival of spring chinook (*O. tshawytscha*) from egg to fry and winter flows. The commercial catches of coho salmon are positively related to annual stream flows, while juveniles inhabited fresh water (Smoker 1953; Scarnecchia 1981). This higher survival of coho was apparently related to higher summer flow for the fry (Neave and Wickett 1948; Neave 1949; Wickett 1951). However, in contrast with coastal streams, in some British Columbia interior streams, winter precipitation is in the form of snow, so that lowest flows are in late winter. In these situations, decreases of water reduce overwintering habitat in side channels, causing high mortality of salmonid fry and parr, due to stranding with subsequent suffocation and freezing of juveniles, low dissolved oxygen levels, and predation (Bustard 1986).

In a boreal river in Quebec, Frenette et al. (1984) investigated relationships between abundance of Atlantic salmon (*Salmo salar*) parr and flow conditions. A positive relationship was found between estimates of parr abundance and previous discharges in late winter and late summer, when the juvenile salmon would have been in the alevin (late winter) or in the underyearling (late summer) stages. Using a multiple linear regression analysis over 7 yr of data, a model for populations of 1+ and 2+ parr was obtained. Parr population estimates were made during years of relatively low densities (Gibson 1978) when density-independent mortality may be relatively more important than density-dependent mortality (Elliott 1984a).

However, in the study by Frenette et al. (1984) the same data were used to develop and test the hypothesis; thus, the levels

TABLE 1. Sources of juvenile Atlantic salmon survival and discharge data from six rivers in Newfoundland and New Brunswick.

River	Year-classes	Spawner index	Parr index	Sources of spawner–parr data	Winter discharge index	Summer discharge index
Western Arm Brook, Nfld.	1972–81	Eggs ($\times 10^3$)	3+ parr (derived from 4+ smolts) from counting fence	Chadwick 1987	Mean February discharges, Ste. Genevieve River near Forresters Point	Mean flow July – August, Ste. Genevieve River
Pollett River, N.B.	1955–62	Potential egg deposition ($\times 10^3$)	1+ parr, 100·yd^{-2} from electrofishing	Elson 1975	Mean discharge in February at Lepreau River	Mean flow July – August, Lepreau River
Northwest Miramichi, N.B.	1952–70[a]	Virtual egg deposition ($\times 10^6$)	1+ parr, 100·yd^{-2} from electrofishing	Elson 1974; Paloheimo and Elson 1974	Mean February discharges for the Little Southwest Miramichi River at Lyttleton	Mean flow July – August, Little Southwest Miramichi River
Miramichi, N.B.	1969–81	Angled kelts	1+ parr, 100·m^{-2} from electrofishing	Chadwick 1985	Mean February discharge Southwest Miramichi River at Blackville	Mean flow July–August, Southwest Miramichi River at Blackville
Restigouche River, N.B.	1971–81	Angled large salmon	1+ parr, 100·m^{-2} from electrofishing	Chadwick 1985	Mean February discharge in the Restigouche River	Mean flow July–August, Restigouche River
Little Codroy River, Nfld.	1954–63	Kelts returning to sea	3+ smolts (plus 2+ smolts × 0.4) from counting fence	Murray 1968a, 1968b; and unpublished data	Minimum mean monthly winter air temperature (°F) for St. Andrews, Nfld.	Mean July–August precipitations (in.), St. Andrews, Nfld.

[a]The year-class of 1951 was not included because there were no winter discharge data for that year, nor the year-classes of 1953 and 1954, which suffered heavy mortality from DDT spraying in those years.

TABLE 2. Regression coefficients and probability levels for one-sided tests that slopes (β, γ_w, γ_s) are less than or equal to zero. Also given are the correlation coefficients between the logarithm of the index of summer discharge (D_s) and winter discharge (D_w).

		Winter flow					Summer flow				
River	$\rho(\ln D_w, \ln D_s)$	α	β	γ_w	$P(\beta \leq 0)$	$P(\gamma_w \leq 0)$	α	β	γ_s	$P(\beta \leq 0)$	$P(\gamma_s \leq 0)$
Newfoundland											
Western Arm Brook	−0.66	6.32	0.46	0.52	0.012	0.011	8.26	0.47	0.54	0.03	0.93
Little Codroy	0.63	6.79	0.36	0.15	0.17	0.25	7.32	0.34	0.007	0.01	0.50
New Brunswick											
Miramichi	0.25	−5.82	0.98	0.097	0.0007	0.25	−7.77	1.03	0.50	0.00025	0.05
Northwest Miramichi	−0.11	1.13	0.88	0.19	0.0014	0.15	0.27	0.90	0.49	0.0008	0.08
Restigouche	0.79	−3.23	0.65	−0.10	0.002	0.682	−3.50	0.63	0.04	0.0034	0.44
Pollett	0.19	1.65	0.47	0.10	0.0021	0.26	−1.28	0.44	0.01	0.0039	0.48

of significance reported are not reliable. We undertook to test the a priori hypothesis that survival of Atlantic salmon eggs and underyearlings is reduced by low river discharge in late winter and late summer.

Methods

We analyzed juvenile salmon survival data from six rivers in Newfoundland and New Brunswick where stock–recruitment relationships were available (Table 1). For each river and for each year-class an index was used of parr abundance, spawner abundance, winter discharge, and summer discharge. The index of spawner abundance was an estimate of egg deposition from fish trap counts when possible, but for some rivers (Miramichi and Restigouche) we had to rely on indices based upon angling data. The parr index was based upon surveys of 1+ parr when possible, but for some rivers (Table 1) our indices were based upon older parr or smolts. Because we are testing hypotheses concerning survival of underyearlings, we are assuming in our regression analysis that there is no correlation between underyearling survival and survival rates of older parr. Mean February discharge and the mean July–August discharge obtained from the nearest unregulated river (Environment Canada 1983) were used as indices of winter and late summer discharges, except for the Little Codroy River where only temperature and precipitation data were used as proxy variables (Murray and Harmon 1969).

The following model was used to test the effect of late summer discharge:

$$P = \alpha S^{\beta} D_s^{\gamma_s}$$

where P is the index of parr abundance, S is the index of spawner abundance, D_s is the index of late summer discharge, and α, β, and γ_s are parameters to be estimated. Our a priori hypothesis was that $\gamma_s > 0$. The model was fitted to the data by log-transforming the three variables and fitting the three parameters by an ordinary least squares regression.

The above a priori regression model was chosen because (i) parr production is a concave increasing function of spawner abundance (Buck and Hay 1984), (ii) the logarithmic transformation helps to stabilize the variance of the model residuals, and (iii) we believed that survival was more likely to be a concave increasing function of discharge than a linear function.

The appropriateness of the regression model was tested by checking for autocorrelation in the residuals (using the Durbin–Watson test), checking for correlations amongst the regressor variables, and graphing residuals to check skewness and constant variance of the model residuals. The residuals were also plotted against the index of spawners to determine if a Ricker-type curve would be more appropriate.

The above procedure was repeated for late winter discharge, where D_s is replaced by an index of late winter discharge, D_w, and the parameters α, β, and γ_w are reestimated.

The assumptions of an ordinary least squares regression could not always be met; therefore, the analysis was repeated using a nonparametric regression which tests whether the dependent

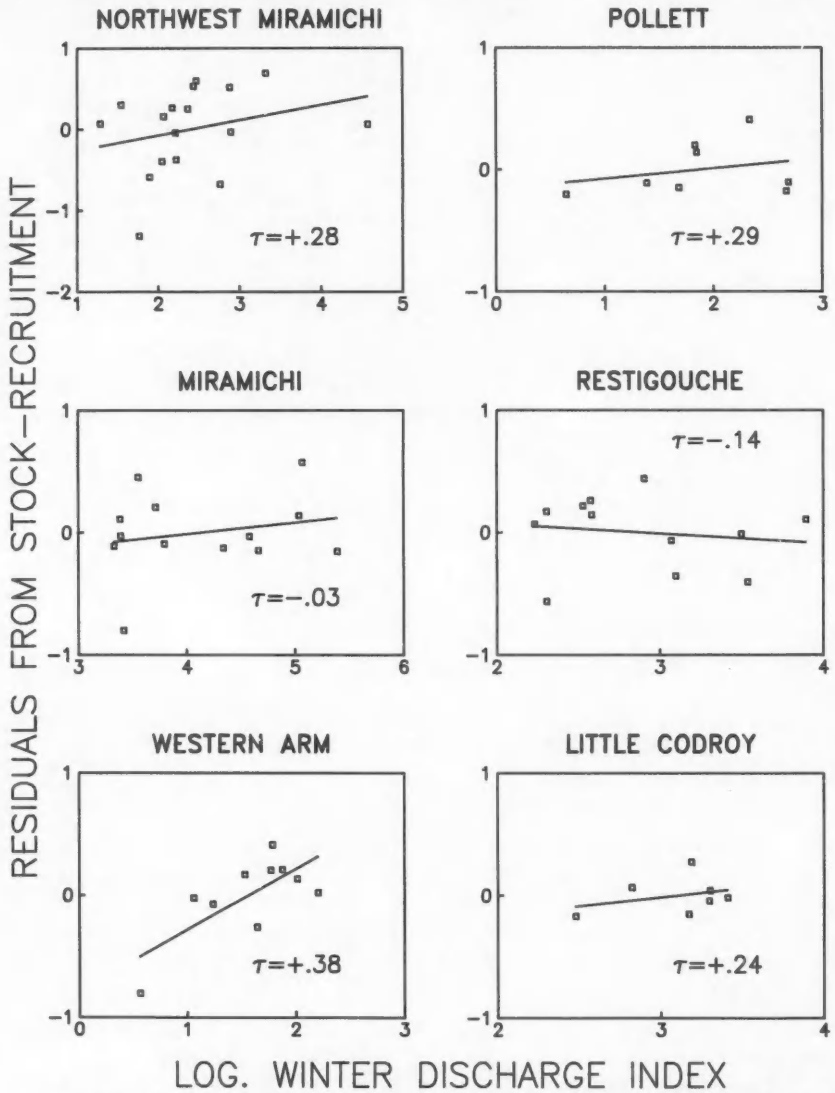

FIG. 1. Residuals from a regression of a stock (spawner index) – recruitment (parr index) relationship. The ordinary least squares regression lines are shown but are sometimes not useful due to heteroscedasticity. Kendall's rank correlation coefficient, τ, is also given.

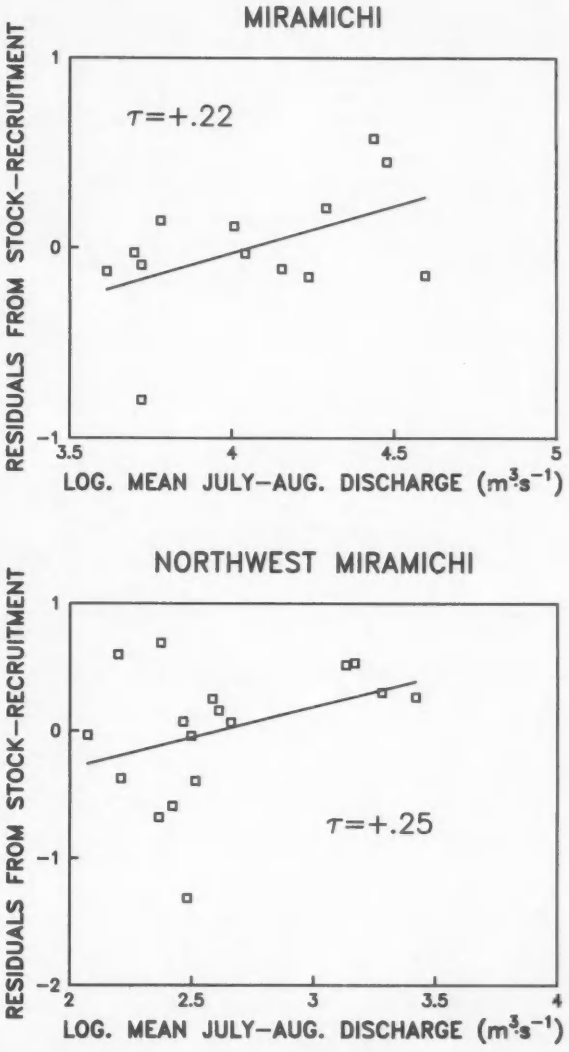

FIG. 2. Same as Fig. 1, except using the summer discharge index.

variable, recruitment, is a monotonically decreasing function of the independent variable, the discharge index. The test we used is the ordering test, which is equivalent to the more familiar Kendall's rank correlation coefficient (Sokal and Rohlf 1981).

We used Fisher's (1954, section 21.1) method of combining probabilities from tests of significance to combine the results for each river to test the above two survival hypotheses. Note that this test is valid only if an a priori decision is made about which significance tests are to be combined. It is also crucial that the individual tests concern the same hypothesis, although the type of data used in the individual tests may vary. See Drinkwater and Myers (1987) for an application of Fisher's (1954) method to a similar problem.

Results

There was no significant autocorrelation among the model residuals, and the logarithmic transformation tended to stabilize the variance. There were positive relationships between winter discharge and survival of 0+ salmon using ordinary least squares regression for all rivers, except the Restigouche (Table 2; Fig. 1). (We used temperature as a proxy variable for winter discharge for the Little Codroy River because discharge data were not available.) The level of significance of all rivers combined led to a rejection of the hypothesis that survival and winter discharge were unrelated ($P < 0.05$).

We could not reject the hypothesis that summer discharge was unrelated to survival. However, there was evidence that summer discharge generally enhances survival for the Miramichi and Northwest Miramichi rivers (Fig. 2).

There was no consistent correlation between summer and winter discharge (Table 2); however, there were positive correlations between the two discharge indices for the Restigouche and the Little Codroy rivers. These correlations should have little effect on our conclusions because the proposed relationships were not strong for these rivers.

Note that the regressions for winter discharge depend strongly on very poor survival during one year with low discharge in the Northwest Miramichi, the Miramichi, and Western Arm Brook (Fig. 1). A nonparametric analysis based upon ranks shows that these low survivals affect our conclusions for one river, i.e. Kendall's $\tau = -0.01$ for the Miramichi. However, these very low survivals appear to be real and asssociated with low discharge. One interpretation of the data presented in Fig. 1 is that winter discharge is only important in years in which discharge is low.

We have least confidence in the data source for the spawner indices of the Miramichi and Restigouche rivers, as this is derived from angling data of kelts or large salmon (Table 1) and is therefore dependent to a large extent on water conditions at the time and on the accuracy of creel censuses. We suspect that the poor results for these two rivers may be associated with unreliable data.

Discussion

Except for the Miramichi and Northwest Miramichi rivers, we found little effect of summer discharge. Unlike the other rivers analyzed, except for the Restigouche River, the Miramichi is a large system and unregulated by lakes, so conceivably would be more severely affected by summer droughts. Low summer flows, however, may have more severe effects on survival of young Atlantic salmon in more temperate or southerly regions of the species' range than in boreal regions. Lishev (1959) suggested that in the Eastern Baltic discharges in summer and winter have positive relationships with survival of underyearlings (summer) or developing eggs (winter), but that summer flows were the major factor. Similarly, Havey and Davis (1970) found in Maine that there was a significant positive relationship between summer rainfall and survival of underyearling landlocked Atlantic salmon. Also, studies in Britain have provided evidence that low summer discharge reduces survival of underyearling brown trout (*Salmo trutta*) (Solomon and Paterson 1980; Elliott 1984a, 1984b). Summer drought and temperature conditions may usually be less severe in boreal regions. However, flow regimes vary between river systems, and depend on river basin morphology as well as climate, and may be regulated considerably by gradient, stream order, groundwater, and the influence of lakes and bogs, etc. (Hynes 1970).

The effects of low winter discharges on survival of Atlantic salmon appear, however, to be more severe in boreal regions

of North America than in temperate regions with milder winters, where such effects have not been documented. In boreal rivers, such as those considered in the present study, the winter climate is relatively severe, and lowest discharges occur in late winter (Frenette et al. 1984). Low winter discharges would reduce percolation of water through the redds and, therefore, oxygen transport to developing eggs and alevins, with subsequent changes in survival (Wickett 1954; Chadwick 1982; Becker et al. 1982). This effect seems to be important only in years of very low winter flow. Also, reduced flows may lower temperatures, and mortality of eggs increases at temperatures below 4°C (Peterson et al. 1977). Winter density-independent effects on developing salmon eggs due to low river discharges therefore need to be considered in development of stock–recruitment models.

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Potential Contribution of Natural Hydrocarbon Seepage to Benthic Productivity and the Fisheries of Atlantic Canada

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Levy, E. M., and K. Lee. 1988. Potential contribution of natural hydrocarbon seepage to benthic productivity and the fisheries of Atlantic Canada. Can. J. Fish. Aquat. Sci. 45: 349–352.

Bacterial processes involving methane and other low molecular weight hydrocarbons may be a substantial contribution to the overall benthic productivity in areas where natural seepage from the seabed occurs. This process may be sufficiently extensive on the continental shelf off the east coast of Canada to be of importance to the fishery.

Les activités des bactéries englobant le méthane et d'autres hydrocarbures de faible poids moléculaire peuvent nettement contribuer à la productivité benthique globale dans des régions où existe une infiltration naturelle venant du fond océanique. Il se peut que ces activités soient suffisamment importantes sur la plate-forme continentale au large de la côte est du Canada pour influer sur la pêche.

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Oceanographic researchers during the past decade have discovered benthic ecosystems that are supported by chemosynthesis (that is, where chemical energy released by the bacterial oxidation of inorganic compounds is used to convert dissolved CO_2 to organic compounds) in environments ranging from the high temperatures and concentrations of H_2S near hydrothermal vents at active rift zones in the deep ocean to the serene conditions typical of passive continental margins. The subject of recent reviews (e.g. Jones 1985; Grassle 1986), these ecosystems pose a serious challenge to a fundamental concept of biological oceanography, that is, that all life in the deep ocean depends on the settling of particulate organic matter which originated in the euphotic zone through photosynthesis. Although this concept so dominated classical marine biology that other mechanisms of carbon fixation have hardly been considered, there is growing evidence that the upward flow of fossil carbon and geochemical energy from natural seepage may constitute a significant, and in some locations perhaps even a dominant, contribution to the support of the benthic community. The potential consequence of this process can be inferred from the many instances world-wide of thriving benthic communities and of commercially valuable fisheries in areas of known natural seeps or oil and gas reserves. This note postulates that natural seepage of hydrocarbons from the continental shelf may be an important factor in the sustenance of the fishery of the east coast of Canada.

Hydrocarbon Seepage and the Support of Benthic Organisms

North Sea — Geophysical data for the Norwegian sector of the North Sea (Hovland and Sommerville 1985; Hovland et al. 1985) indicated the presence of plumes of gas bubbles in the water column and of gas in the sediments above a salt diapir which, over geologic time, was forced upwards through the overlying hydrocarbon-bearing sedimentary rocks, creating a system of fissures through which the gas escapes. Observations with an unmanned submersible showed that the gas was streaming from small vents in the seafloor and small funnel-shaped "craters" had formed in the sediment around each vent. The gas was almost pure methane, but the sediments contained hydrocarbons ranging in molecular weight to hexane. In contrast with the generally barren seafloor, there were sharply defined "lush islands" with a rich variety of benthic flora and fauna and accumulations of shell debris. Accordingly, Hovland et al. (1985) surmised that the hydrocarbon-bearing sediments presented "a localized physiochemical environment which stimulates marine life" and that "filter-feeding animals such as bivalves, then feed on the bacteria and they, in turn, are eaten by animals further up the food chain."

In the United Kingdom sector, gas was detected (Hovland and Sommerville 1985) at a large "pockmark," a depression formed in the seafloor by releases of gas from soft, fine-grain sediments. Toward the center of the pockmark and beneath the thin covering of sediment there was a crust of a light-colored, rock-like material consisting of sand, silt, and shell debris cemented together by calcite and aragonite. Ratios of the stable carbon isotopes indicated that the carbonate was derived from methane seeping through the seafloor. The preferential dissolution of aragonite accounted for the weathered appearance of the exposed upper surface of the concrete-like slabs which teemed with life. Molluscs, crabs, and starfish were much more abundant near the seep than elsewhere.

Baffin Island Continental Shelf — Chemical and geological studies (MacLean et al. 1981) suggest that natural seepage of gas and oil from a hydrocarbon-bearing structure underlying the Baffin Island continental shelf occurs along the contact between a Precambrian basement high and the adjoining Tertiary/Pretertiary sediments. Visual examination of the seafloor from a manned submersible in 1981 revealed the presence of

patches of "whitish growth" and a dark-grey to black discoloration suggesting anoxic conditions in the underlying sediment (MacLean 1982). Further study in 1985 (Grant et al. 1986) identified the white substance as mats of bacteria, predominantly *Beggiatoa*, and shipboard microbiological studies demonstrated the dark uptake of CO_2 and its enhancement with the addition of thiosulfate, the incorporation of thymidine into DNA (heterotrophic production), and the uptake of organic substrates (glutamic acid and hexadecane). Gas collectors placed on the seafloor proved that methane, ethane, and hydrogen sulfide were escaping from the sediments. Thus, all the components required for chemosynthetic primary productivity were present, and the experiments indicated that both chemolithotrophic and chemoorganotrophic processes possibly occur at the seabed. In addition, organic chemosynthetic primary productivity by which energy derived from the bacterial oxidation of methane and other low molecular weight hydrocarbons is used to fix dissolved CO_2 is a distinct possibility.

These processes, all of which entail the upward flow of fossil carbon and geochemical energy, could account for the observed distribution of benthic organisms near the seep at Scott Inlet. On what were ostensibly identical sediments, this ranged from very sparse on a seemingly barren seafloor to "oases" with rich stands of crinoids and dense populations of shrimp- and crab-like animals. The benthic community was dominated by active suspension feeders at the sediment–water interface and in the water column immediately above, while detrital feeders in the sediments were noticeably absent. Such a distribution is incompatible, with the major source of sustenance being the diffuse descent from the euphotic zone of organic matter of photosynthetic origin. Numerous depressions in the sediments, similar to the craters described by Hovland et al. (1985), were also observed.

The patches of bacterial mat at Scott Inlet were often associated with a hard, cemented crust which was covered with a thin layer of fine sediment. In one location, the mats were found in a depression thought to be a pockmark, and closer inspection revealed narrow fissures which extended into the seafloor and may have served as a route for egress of hydrocarbons from the underlying sediments. Although no flow of gas or oil from the fissures was observed, oil escaped from beneath a piece of the crust when it was broken off and drained from it after recovery at the surface. Since the crust was indurated bottom sediment and small pebbles of igneous rock cemented with carbonate, it appears that seepage of brine was also occurring, with the calcium in the brine reacting with dissolved CO_2 to precipitate calcium carbonate and cement the adjoining sediment into the crust. Similar crusts have been reported at methane seepages in the North Sea (Hovland and Sommerville 1985) and in the Oregon subduction zone (Kulm et al. 1986).

Whatever the mechanisms, there was a definite association at Scott Inlet between the bacterial mats, the suspected pockmark, the escaping gaseous and liquid hydrocarbons, the hydrogen sulfide, and carbonate crust, and the distribution of the benthic organisms. Processes occurring at the seafloor seem to make a major contribution to the total annual supply of organic carbon that is available to the benthic community on this portion of the Baffin Island shelf. Taking account the likelihood of hydrocarbon seepage at other locations along the Baffin Island shelf (Levy 1981), the proven hydrocarbon resources of the Beaufort Sea, and the potential reserves of Lancaster Sound and the Canadian archipelago, and considering that the low light intensities and the heavy sea ice during much of the year restrict photosynthetic productivity in the water column, it is conceivable that hydrocarbon seepages represent a major component of the total annual productivity in the Canadian arctic.

Other areas — Both gas and oil seep from the seafloor of the Louisiana slope, and ratios of stable carbon isotopes demonstrated that the filter-feeding benthos feeds on isotopically light bacteria rather than organic particles of recent photosynthetic origin (Kennicutt et al. 1985). Mussels from this site consume methane at a very high rate, with the activity occurring only in the bacteria-containing gill tissue and the isotopically light carbon then being distributed throughout the animals (Childress et al. 1986). Similarly, the extremely high proportion of isotopically light carbon ($\delta^{13}C$ values as low as $-74‰$) in mussels collected at the Florida Escarpment (Paull et al. 1985) and the presence in their gill tissues of subcellular inclusions (thought to be symbionts) as well as the enzyme systems for methylotrophic metabolism (Cavanaugh et al. 1987) provide further evidence that the oxidation and incorporation of carbon from methane represents a major nutritional component for these animals.

Similarly, despite high concentrations of potentially toxic materials, macrofaunal densities at an oil seep off the coast of California were considerably higher than those at comparable sites nearby (Davis and Spies 1980). The benthos was characterized by mats of *Beggiatoa*, a food source for the invertebrates, and carbon isotope data indicated that fossil carbon was being incorporated into the coastal food web via the decomposition of petroleum by heterotrophic bacteria (Spies and DesMarais 1983). Detailed study of the rates of sulfate reduction, oxygen fluxes, and hydrocarbon degradation (Montagna and Spies 1985; Montagna et al. 1986) indicated that petroleum-derived microbial carbon was an important supplement to the benthic invertebrate productivity. Further, isotopically light carbon from the methane-rich porewater expelled by the compressive forces in the Oregon subduction zone is present in the benthos and becomes incorporated into the food chain (Kulm et al. 1986).

Potential Contribution of Hydrocarbon Seepage to Fisheries of Atlantic Canada

The association between benthic organisms and the natural seepage of hydrocarbons, the presence of isotopically light carbon in seep invertebrates, and various experiments with the animals themselves demonstrate that fossil carbon can be incorporated directly into the benthic food web. Thereby, petroleum-related substances can play a beneficial role in seep areas, although the mechanisms are not fully understood. One process, the direct uptake of seep hydrocarbons and incorporation into bacterial biomass, which is then available to higher members of the food web, has been established. A second, chemautotrophy, may occur, since H_2S is often present in brines accompanying the hydrocarbon seepage or produced under the anoxic conditions generated in the sediments by the microbial oxidation of hydrocarbons. Bacteria with the requisite enzyme systems to oxidize the H_2S can then drive the Calvin-Benson cycle. Third, it is conceivable that organic chemosynthesis, where bacterial oxidation of hydrocarbons is the source of chemical energy, occurs. Whatever the mechanism, there is considerable evidence that the usual biogeochemical process can be circumvented in areas of hydrocarbon seepage and be replaced by a short and highly efficient food chain based on

bacterial conversion of fossil hydrocarbons to biomass, which then provides direct support to the benthos.

Although the beneficial effect of natural hydrocarbon seepage is highly localized, in some areas there are sufficient numbers of seeps that, collectively, the upward flow of geochemical carbon and energy provides a substantial portion of the sustenance of the benthos. Where other factors are favorable, this contribution to the total benthic productivity seems sufficient to be reflected in the fishery. Hovland et al. (1985) provided evidence that this is the case in the North Sea, and there is considerable evidence that natural seepage makes a sufficient contribution to the overall pool of organic carbon available to the invertebrates and prey species which maintain the valuable fisheries on the Labrador Shelf, the Grand Banks of Newfoundland, the Scotian Shelf, and on Georges Bank. All of these areas not only support commercially important fisheries but also have proven or potential reserves of hydrocarbons as gas, condensate, or oil, and while natural seepage has not been unequivocally demonstrated in every case, there is evidence that it does occur. For example, a survey of the Labrador Shelf demonstrated that the concentrations of petroleum residues in the surficial bottom sediments were highest at the edge of the shelf off Nain Bank and in the Labrador Marginal Trough, especially in the Hopedale Saddle area (Levy 1986). While active seepage was not observed, the boundary between the Precambrian rock comprising the coast and shelf inshore of the Marginal Trough and the semiconsolidated Tertiary sedimentary rocks to the seaward (Grant 1972) provides a potential route for seepage from the underlying hydrocarbon-bearing rocks.

Similarly, although a brief chemical survey did not provide conclusive evidence of seepage on the Grand Banks (Levy 1983), gas-charged sediments and patches of "white slime" were observed on the seafloor during geological investigations of the Hibernia area and a gas "leak" has been reported on the northeast slope of the banks nearby (Fader 1985). Such leaks may be much more common than is presently realized, as gas is present at 300–500 m below the seabed in the Tail of the Banks area (Fader and Miller 1986). It is conceivable that the large areas of shell fragments and thriving benthic communities on the Grand Banks are derived, in part, from relationships between hydrocarbon-utilizing bacteria, molluscs, and other animals. Since seep invertebrates flourish during periods of active seepage, but suffer mass mortality when it ceases, such interruptions could account for the distribution of the shell beds. Although pockmarks are not present on the banks because the regime is too dynamic for the accumulation of fine sediments, they do occur in the quiescent areas of the deeper channels (Fader et al. 1982). Pockmarks are particularly abundant on the Scotian Shelf, and in places, relic pockmarks occupy as much as 18% of the total seafloor (Josenhans et al. 1978). As is the case on the Grand Banks, sidescan sonograms indicate the presence of shell beds on the Scotian Shelf. Both areas not only have proven reserves of hydrocarbons but support valuable fisheries.

Finally, on Georges Bank where there are extremely valuable fisheries for scallops and groundfish and there is sufficient potential for petroleum that exploratory drilling is being considered, remarkably high concentrations of methane and other low molecular weight hydrocarbons were found in surficial bottom sediments during a recent survey (unpublished data). Thus, the unique population of scallops on Georges Bank may be the result, in part, of relationships between the animals and seep-adapted bacteria that use hydrocarbons from natural seepage to augment the supply of organic carbon from photosynthesis. Indeed, associations between bacteria and the dominant macrofaunal organisms might very well be a factor in determining the high population densities of commercially valuable species in many places on the Canadian continental shelf. Since biodegradation has the additional effect of "detoxifying" the environment in regions where natural seepage occurs and the benthos has successfully adapted to and thrives under conditions that would normally be detrimental or even intolerable, bacterial processes can readily account for the many examples globally where lucrative fisheries exist in areas of petroleum reserves. Such trophic interactions may be the reason that it has not been possible in the past to demonstrate the anticipated deleterious effects of oil "pollution" on fish stocks and on fisheries.

Concluding Remarks

If the concept that benthic communities in areas of natural seepage derive a substantial portion of their sustenance from hydrocarbons through the dual role of chemosynthesis and biodegradation proves to be valid, it is evident that the fisheries of the Canadian east coast may not be as rigidly coupled to photosynthesis by phytoplankton and the cycling of inorganic nutrients as has been the doctrine in the past. Consequently, the tenet that primary photosynthetic productivity controls the potential for fish production might be seriously deficient. A troublesome problem with this concept has been the inability to account for the carbon required to support benthic communities; for example, using presently accepted techniques, Smith (1987) demonstrated that the amount of carbon caught in sediment traps in the Pacific Ocean was consistently less than the amount being utilized by the benthos and therefore concluded that sources of carbon in addition to the rain of organic detritus must be available to the benthos. Because of the difficulty in balancing carbon budgets, it has been necessary to invoke processes by which carbon may be accumulated, imported onto, or exported from the continental shelves, as appropriate. Clearly, existing benthic models should be reconsidered to include the upward flow of fossil carbon and geochemical energy from the underlying sediments as a possible component of the "missing" carbon. For the rational management of fisheries, it is evident that this and other mechanisms of carbon fixation must be taken into account as an important supplement, or perhaps in some environments as an alternative, to photosynthesis. This concept may also be a key factor in assessing the potential impact of offshore exploration and the production of oil and gas; that is, by considering photosynthesis and chemosynthesis together, a solution may be offered both to the long-standing problems associated with the management of the fishery and to the stalemate regarding the coexistence of the fishing and offshore oil industries — two industries that have all too often been assumed in the past to be incompatible.

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Evidence for Antifreeze Protein Gene Transfer in Atlantic Salmon (*Salmo salar*)[1]

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Fletcher, G. L., M. A. Shears, M. J. King, P. L. Davies, and C. L. Hew. 1988. Evidence for antifreeze protein gene transfer in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 45: 352–357.

Atlantic salmon (*Salmo salar*) freeze to death if they come into contact with ice at water temperatures below −0.7°C. Consequently, sea-pen culture of this species in cold water is severely limited. Winter flounder (*Pseudopleuronectes americanus*) survive in ice-laden seawater by producing a set of antifreeze polypeptides (AFP). We are attempting to make the Atlantic salmon more freeze resistant by transferring antifreeze protein genes from the winter flounder to the genome of the salmon. Salmon eggs were microinjected with linearized DNA after fertilization. Individual fingerlings (1–2 g) were analyzed for flounder AFP genes by genomic Southern blotting. DNA from 2 out of 30 fingerlings showed hybridization to the flounder DNA probe. Hybridization bands following cleavage by restriction enzymes Sst I and Bam HI were identical to those of the injected DNA.

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Hybridization following Hind III digestion indicated that the flounder AFP gene was linked to the salmon genome. These hybridization signals were absent in the DNA from control fish. The intensity of the hybridization signals indicated that there was on average at least one copy of the AFP gene present per cell.

Le saumon de l'Atlantique (*Salmo salar*) meurt gelé s'il vient en contact avec de la glace quand la température de l'eau est inférieure à −0,7°C. Ceci limite sévèrement l'élevage de cette espèce en cages mouillées en eau froide. La plie rouge (*Pseudopleuronectes americanus*) peut survivre en eau chargée de glace en produisant une série de polypeptides antigel (AFP). Les auteurs tentent de rendre le saumon de l'Atlantique plus résistant au gel en transférant des gènes de la protéine antigel de la plie rouge au génome du saumon. Après leur fertilisation, des oeufs de saumon ont été injectés d'ADN linéarisé. À l'aide de la technique de tamponnement génoimique « Southern », on a tenté de relever la présence de gènes AFP de la plie rouge chez les tacons O+ (1–2 g). L'ADN de 2 des 30 tacons examinés a montré une hybridation avec la sonde radioactive d'ADN de la plie rouge. Des bandes d'hybridation suite au clivage par les enzymes restrictifs Sst I et Bam HI étaient identiques à celles de l'ADN injecté. L'hybridation suite à la digestion de Hind III révèle que le gène AFP de la plie rouge était lié au génoime du saumon. Ces signes d'hybridation étaient absents chez l'ADN des poissons témoins. L'intensité des signes d'hybridation révèle qu'il existait en moyenne au moins une copie du gène AFP par cellule.

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It is becoming increasingly apparent that culture of salmonids, particularly Atlantic salmon (*Salmo salar*), is feasible and economically desirable in Atlantic Canada (Aiken 1984). However, one of the major problems associated with the development of sea-cage culture of salmon in Atlantic Canada is the subzero winter water temperatures which prevail throughout most of the usable coastline (Saunders 1986; Aiken 1986). Atlantic salmon freeze to death when the temperature falls below −0.7°C (Saunders et al. 1975). Therefore, inshore salmonid culture is restricted to a very few areas where the water temperatures do not decline below 0°C (Sutterlin et al. 1981).

It has been well established that marine fish that survive icy water do so by synthesizing antifreeze proteins or glycoproteins and secreting them into their body fluids (Hew and Fletcher 1985; Scott et al. 1986). These antifreezes, which act noncolligatively, depress the freezing temperature of the body fluids and thus the freezing temperature of the fish to safe levels. No evidence for the presence of antifreeze peptides has been found in any salmonid (*Salmo salar, Salmo trutta, Salmo gairdneri, Salvelinus fontinalis, Salvelinus alpinus*, G. L. Fletcher, unpubl. data).

During the past 10 yr, we have carried out extensive studies of the antifreeze polypeptides (AFP) and their genes from the winter flounder (*Pseudopleuronectes americanus*) which produces a family of at least seven AFP during the winter. These peptides are α helices rich in alanine (60 mol%) which range from 3300 to 4500 in molecular mass (Fletcher et al. 1985; Hew and Fletcher 1985).

The AFP genes from the winter flounder consist of a gene family with 30–40 members, approximately two thirds of which are arrayed in direct tandem repeats (Scott et al. 1985), with the remainder linked but irregularly spaced (Davies et al. 1984). The tandemly linked AFP genes code for the major AFP components found in flounder plasma (Scott et al. 1987).

A recent study has demonstrated that AFP can confer increased freezing resistance to salmonids (Fletcher et al. 1986). Intraperitoneal injections of winter flounder AFP improved the freezing resistance of rainbow trout in direct proportion to the concentration of AFP in the blood, thus indicating that there are no species-specific AFP protein receptors. These results suggest that the Atlantic salmon will tolerate subzero water temperatures and ice if the winter flounder AFP gene can be inserted into the salmon genome and expressed in meaningful quantities.

We report here a successful first step towards producing transgenic salmon with increased freeze resistance by demonstrating the presence of winter flounder AFP genes in Atlantic salmon developed from microinjected eggs.

Materials and Methods

Atlantic salmon adults (3–5 kg) were captured from the Exploits and Colinet river sytems (Newfoundland) and transported live to the Marine Sciences Research Laboratory 2–3 wk prior to spawning. At the laboratory, male and female salmon were maintained at seasonally ambient photoperiod in 2 × 2 × 0.5 m deep aquaria supplied with flowing freshwater and air.

Eggs and sperm were stripped from salmon which had been anaesthetized in a dilute solution of *t*-amyl alcohol. The eggs were stored up to 3 d in plastic containers at 4°C until use. Sperm were stored in 20-mL vials and checked for motility under a compound microscope just prior to fertilizing the eggs.

Eggs were fertilized up to 2 h prior to microinjection and rinsed with several changes of ice-cold (4°C) salmon Ringer. When this procedure is used the eggs are fertilized but not activated. Activation occurs when the eggs are placed in a hypotonic solution (Ginsberg 1963).

Preparation of DNA — The DNA used for microinjection was the genomic subclone 2A-7 which contained a 7.8-kb Bam HI fragment of winter flounder DNA cloned into the Bam HI site of plasmid pUC 9 (Scott et al. 1985) (see Fig. 2). This fragment was derived from a λ charon 30 flounder genomic DNA library and represents one of the tandem repeats that each contain one copy of the 1-kb AFP gene. The 2A-7 subclone was prepared by banding in CsCl/ethidium bromide and was linearized by digestion with Eco RI. The digested DNA was extracted with phenol/chloroform, recovered by ethanol precipitation, and stored frozen in 10 m*M* Tris–HCl (pH 7.4)/1 m*M* EDTA.

Microinjection — The injection apparatus consisted of a glass needle 3–5 μm (outside diameter) driven by a micromanipulator. The volume of DNA solution injected (2–3 nL) was regulated using short bursts (100–1000 ms) of N_2 at 200 kPa. The timing of the bursts was controlled using a Grass 544 Stimulator (Grass Instruments, Quincy, MA). Eggs were injected with ~ × 10^6 copies of linearized clone 2A-7 through the micropyle within 3 h of fertilization and incubated in freshwater at 8°C. The micropyle, which is located in close proximity to the egg nuclear area, was visualized using a dissecting microscope at 20× magnification (Ginsberg 1968; Riehl 1980; Pringle 1987).

Analysis of hatchlings — Thirty fingerlings (1–2 g each) collected 8 mo after injection were frozen in liquid N_2 and stored at −70°C for analysis. Genomic DNA was prepared from individual fingerlings by the method of Blin and Stafford (1976), as modified by Scott et al. (1985). To test for the incorporation of the flounder AFP gene, salmon genomic DNAs (15 μg) were digested with a threefold excess of restriction enzymes for 3 h at 37°C, electrophoresed in 0.7% agarose gels, and blotted onto either nitrocellulose or Biotrace (Southern 1975). Southern blots were probed with a gel-purified, nick-translated 2.7-kb Sst I fragment from subclone 2A-7 that completely encompasses the AFP gene. Blots on Biotrace were stripped of probe after autoradiography and were rehybridized with nick-translated pUC-9.

Results

Of the 1800 Atlantic salmon eggs injected with DNA, approximately 80% survived to hatching. This survival rate is essentially the same as that of uninjected eggs. For preliminary screening, genomic DNAs were digested with Sst I and Bam HI and Southern blotted. Two DNAs (26 and 36) from the 30 individual fingerlings tested showed hybridization signals with the AFP gene probe in an initial screening (not shown). These two DNAs were retested along with a negative control (45, one which showed no hybridization signals) and again showed hybridization to the probe (Fig. 1A). The Sst I digests (*S*) of 26 and 36 both resulted in a band of hybridization at ~2.7 kb, which corresponded to one of the major Sst I fragments that hybridized in winter flounder (wf) genomic DNA. This is in effect identical to the Sst I fragment present in subclone 2A-7 (Fig. 2) and referred to as the "b" polymorphism in Scott et al. (1985) which was used as the hybridization probe in Fig. 1A. This hybridization signal is not seen in the control (45), even though the ethidium bromide stained pattern of mass bands indicated that this DNA has been digested to the same extent as 26 and 36 (not shown). Digestion of 26 and 36 with Bam HI (B) produced a main band of hybridization at 7.8 kb (Fig. 1A), consistent with predictions from the map in Fig. 2, and several minor bands. These two samples differed in their hybridization pattern after Hind III digestion. In 26 the major band of hybridization appeared at 13.5 kb, while in 36 it was 9.4 kb. As in the Bam HI lanes, there were several minor bands of hybridization. Finally, in the undigested samples the only hybridization signal coincided with the band of high molecular weight genomic DNA.

To study the fate of the plasmid vector which remained attached to linearized 2A-7, the blot shown in Fig. 1A was stripped of hybridization probe and reprobed with labelled pUC 9 (Fig. 1B). There is a significant amount of hybridization to the undigested DNA in the control lanes of 26 and 36 but no bands of hybridization below this high molecular weight material and only background hybridization to the undigested flounder DNA or 45 DNA. Several bands of hybridization were present in all three enzyme digests of samples 26 and 36 which were absent from the single digest of 45. The length of these bands generated with each enzyme were different from one DNA to the other as they would be if they represented independent integrations into the genome.

Discussion

The hallmarks of a stably integrated copy of the winter flounder AFP gene derived from linearized 2A-7 can be predicted from Fig. 2. In all transgenic fish, Sst I and Bam HI should liberate the gene in 2.7- and 7.8-kb restriction fragments, respectively. Hind III should give a gene-containing fragment of at least 7.8 kb, but with a varied upper length. The latter prediction holds because there is only a single Hind III site in 2A-7 in the multiple cloning site of the pUC 9 portion. If a single copy of 2A-7 is integrated, then the second Hind III site must come from the flanking salmon genomic DNA. When the same digests are probed with the 2.7-kb pUC 9 plasmid, they should show an entirely different pattern of digestion, with minimum lengths for the hybridization signal of 2.7 kb in both the Bam HI and Hind III digests and a minimum of ~4.7 kb in the Sst I digest. All these criteria were met for fish 26 and 36.

The most likely explanation for the minor bands in the Bam HI and Hind III lanes of 26 and 36 is that they represent cleavage products of 2A-7 that have been independently incorporated into the salmon genome. In this situation, one would expect to see fewer of these accessory bands in the Sst I lanes than in the other two digests, simply because there is less chance of the breaks in 2A-7 occurring within the central 2.7-kb Sst I fragment than within the 7.8-kb Bam HI fragment. When the overall hybridization signal present in 26 and 36 is compared with the signal derived from the 30–40 genes in the Sst I digested winter flounder standard (Fig. 1A), it is clear that more than one copy of the AFP gene had been incorporated into the transgenic salmon. The exact number of copies per cell must await further research.

The integration frequency of the injected genes in the present study (2 out of 30 (6%)) appears to be similar to that observed by other investigators using microinjection procedures. Hammer et al. (1985) found an integration frequency of 1–13% for several mammals. Maclean et al. (1987) reported a 5% integration frequency of rat growth hormone – mouse metallothionein fusion gene in the genome of rainbow trout. Similarly, Dunham et al. (1987) demonstrated, using restriction analysis, the successful integration of a mouse metallothionein – human growth hormone fusion gene in 2 out of 10 (20%) channel catfish. Zhu et al. (1985) reported that 50% (3 out of 6) of their goldfish injected with a human growth hormone gene construct were transgenic, while Chourrout et al. (1986) claimed an integration frequency of 75% by injecting a human growth hormone cDNA sequence into rainbow trout eggs. Although Chourrout et al. (1986) found evidence for the growth hormone gene in the high molecular weight DNA, restriction analysis was not carried out. Therefore, unequivocal evidence of integration is lacking.

Winter flounder AFP genes have been successfully transferred to *Drosophila melanogaster* (Rancourt et al. 1987). The central 2.7-kb Sst I fragment of 2A-7 was incorporated into

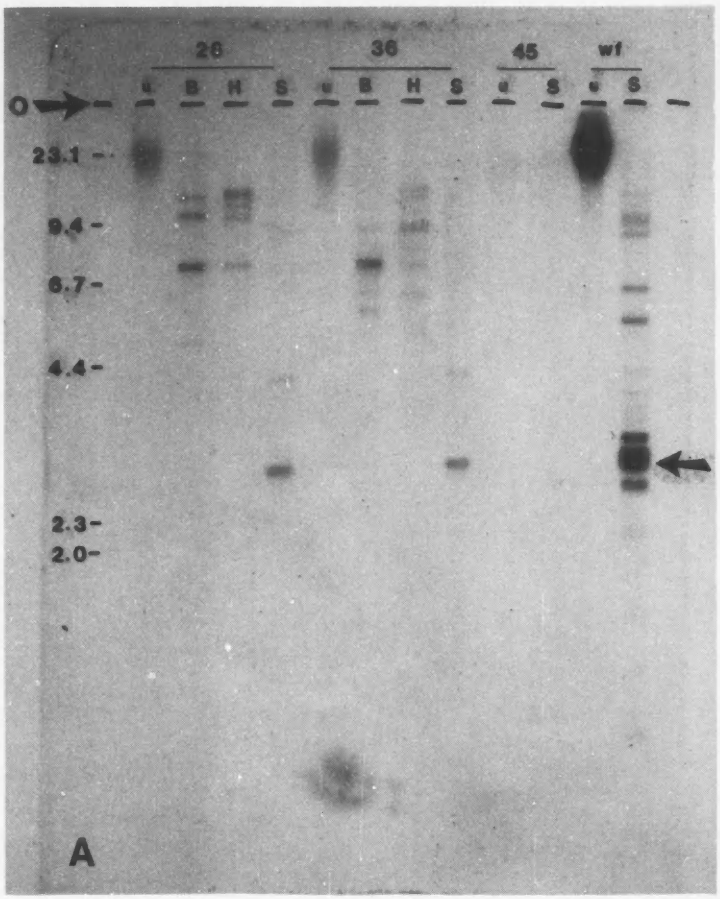
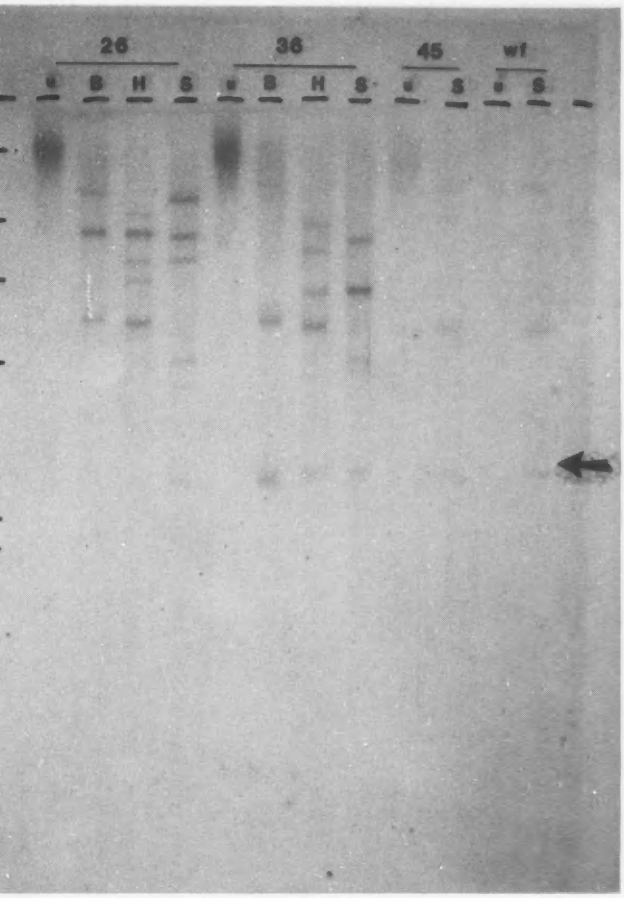


FIG. 1. Genomic Southern blots of salmon DNAs. DNAs (15 μg) are from salmon 26, 36, and with restriction enzymes Bam HI (B), Hind III (H), and Sst I (S). The origin of the gel (O) is inc (Hind III digested DNA) are indicated below. On the right-hand side, the arrow indicates the po in flounder DNA that matches the Sst I fragment in the plasmid 2A-7. (A) The blot was probe from 2A-7 as described by Scott et al. (1985); (B) the same blot was reprobed with nick-transl



, 36, and 45 and winter flounder (wf) undigested (u) and digested
O) is indicated by an arrow, and the lengths (kb) of DNA standards
s the point of migration of the AFP gene-containing Sst I fragment
s probed with nick-translated AFP gene-containing Sst I fragment
ck-translated pUC-9.

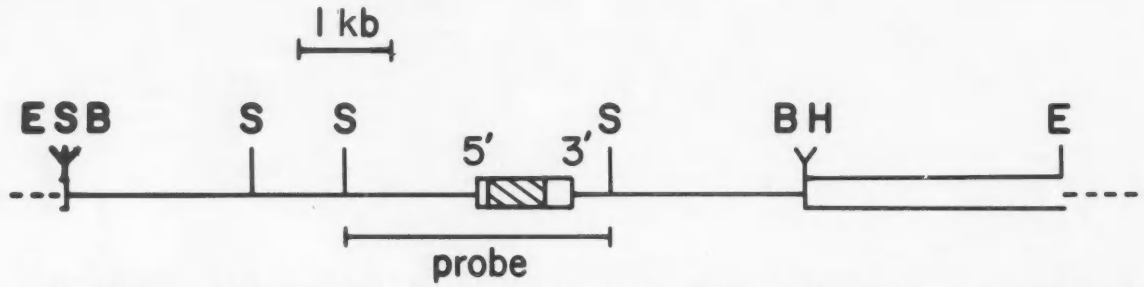


FIG. 2. Restriction map of the integrated, linearized plasmid 2A-7. Plasmid 2A-7 (Scott et al. 1985), linearized at its unique Eco RI site (E), is shown integrated into salmon genomic DNA (broken line). The pUC-9 section of 2A-7 is indicated by double lines and the flounder DNA insert by a single line within which is located a rectangular box representing the 1-kb AFP gene. The open areas of the box are the exons and the hatched area is the intervening sequence. The cleavage sites of Bam HI (B), Sst I (S), and Hind III (H) are marked. The 2.7-kb Sst I fragment used as a probe is underlined.

the *Drosophila* genome using the transposable "P" element vector. In this evolutionary distinct organism there is no expression of the native flounder AFP gene. However, expression was achieved by incorporating a *Drosophila* heat shock (Hsp-70) promoter upstream of the 5′ end of the AFP gene. In this situation, all aspects of expression, including polyadenylation and splicing of the pre-mRNA and export of the AFP to the hemolymph, were successful.

The results of this study strongly suggest that the AFP DNA microinjected into Atlantic salmon eggs was stably integrated into the salmon genome. Clearly, however, irrefutable evidence for this must await the transfer of these genes to progeny. Moreover, in order for such transgenic fish to be of value to the aquaculture industry the AFP genes must be expressed in meaningful amounts. In this regard it should be noted that the 2A-7 subclone used in this study is an entire tandem repeat of the winter flounder antifreeze gene and should therefore contain the DNA sequences responsible for controlling gene expression. During the upcoming year, surviving salmon from the present series of experiments should approach a size where small samples of blood can be obtained from individually tagged fish. Genomic Southern blot analysis can be done on DNA from red blood cells, while the plasma can be assayed for the presence of antifreeze proteins. In this way, transgenic salmon that actively express the AFP genes can be selected for future breeding experiments.

Acknowledgments

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Bacterial Hydrolysis of Urea in the Tissues of Carcharhinid Sharks

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Knight, I. T., D. J. Grimes, and R. R. Colwell. 1988. Bacterial hydrolysis of urea in the tissues of carcharhinid sharks. Can. J. Fish. Aquat. Sci. 45: 357–360.

Ureolytic activity was detected in liver homogenates of two carcharhinid shark species, *Galeocerdo cuvieri* and *Negaprion brevirostris*, employing ^{14}C-labeled urea to monitor mineralization over 48 h. Significant ($P < 0.05$) urea hydrolysis was detected in liver homogenates within 48 h whereas no hydrolysis was detected in homogenates incubated with either ampicillin or the vibriostatic agent O/129, indicating bacterial mediation of the ureolytic activity. Urea hydrolysis could not be detected in blood samples. The autochthonous microflora of sharks has been found to contain ureolytic bacteria, and the present study supports the conclusion that they are present in organ tissues of healthy sharks but are not present in the blood.

On a découvert une activité uréolytique dans des homogénats de foie de deux requins, soit *Galeocerdo cuvieri* et *Negaprion brevirostris* (Carcharhinidae); la minéralisation a été suivie pendant 48 h à l'aide d'urée marquée au ^{14}C. On a noté un niveau élevé d'hydrolyse de l'urée ($P < 0,05$) chez les homogénats de foie en dedans de 48 h tandis qu'aucune hydrolyse n'a été relevée chez des homogénats incubés avec de lampicilline ou du O/129, un agent vibriostatique. Ceci révèle que les bactéries servent de médiateur de l'activité uréolytique. Aucune hydrolyse de l'urée n'a été notée dans les échantillons de sang. On a découvert que la microflore autochtone des requins contient des bactéries uréolytiques et la présente étude étaye la conclusion qu'elles sont présentes dans les tissus organiques de requins en santé mais non dans le sang.

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Urea hydrolysis in shark tissue, first described by Wood (1950), was reported by Simidu and Oisi (1951) to be associated with bacterial spoilage and not a result of constitutive shark urease activity. In studies of the normal flora of neritic sharks inhabiting shallow waters near Bimini, Bahamas, 65% of all bacterial isolates were found to be urease positive (Grimes et al. 1985). Among the urease positive isolates were several species of the genus *Vibrio*, for which the ability to hydrolyze urea is unusual (West et al. 1986).

The isolation of bacteria from the internal tissues, organs, and body cavities of sharks, areas that are normally sterile in most animals, led us to hypothesize that ureolytic bacteria play a role in controlling shark tissue urea storage and flux. In the studies reported here, radiolabeled urea was used to determine whether bacterial hydrolysis of urea occurred in the internal tissues of free-living sharks.

Materials and Methods

Sample collection — Two sharks (Table 1) were collected at Bimini, Bahamas, in May 1986 and dissected aseptically in the laboratory aboard the R/V *Columbus Iselin*. Both sharks were captured on longlines, employing frozen Boston mackerel and fresh barracuda as bait and set near shore at depths of 3–5 m. The sharks were transported live to the R/V *Columbus Iselin* in a skiff and maintained aboard ship in a seawater holding tank for 30 min to 1 h before sacrificing and dissection.

The following samples from each shark were aseptically removed for analysis: blood (collected via the caudal vein), liver, kidney, and muscle, the latter from the dorsoventral

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TABLE 1. Characteristics of sharks used as source of tissue for ^{14}C-labeled urea mineralization studies.

Species	Common name	Sex	Weight (kg)	Total length (cm)	ID No.
Negaprion brevirostris	Lemon shark	Female	105	268	1
Galeocerdo cuvieri	Tiger shark	Female	7	134	2

region of the animals. The ventral region of each animal was thrice scrubbed with antiseptic (Betadine) prior to making incisions and care was taken to maintain asepsis during surgery.

Sample preparation — A one-half equivalent volume of sterile 0.1 *M* phosphate-buffered saline (0.85% NaCl) (PBS), pH 7.3, was added to each sample after it had been weighed. The samples were homogenized using an Omnimixer (Sorvall, Washington, DC). Because the muscle homogenate was highly viscous, an additional one-half equivalent volume of PBS was added to the muscle samples. Each tissue homogenate was divided into three subsamples, to each of which was added a one-third volume of one of the following reagents: sterile saline (1.5% NaCl); 2,4-diamino-6,7-diisopropyl-pteridine (vibriostatic agent O/129; Sigma Chemical Co., St. Louis, MO), 900 μg/mL in 1.5% saline; or ampicillin (sodium salt), 1200 μg/mL in 1.5% saline. Each treated homogenate was thoroughly mixed by vortexing. Final homogenate concentrations of O/129 and ampicillin were 225 and 300 μg/mL, respectively.

Treated homogenates were incubated with ^{14}C-labeled urea using a modified version of the incubator and trap system described by Somerville et al. (1985). A 4-mL, uncapped, polypropylene omnivial (Wheaton Industries, Millville, NJ) was trimmed to fit inside a borosilicate scintillation vial (American Scientific Products, McGraw Park, IL) which served as the reaction vessel. Vessels were sealed with an open top screw cap fitted with a septum liner. After autoclaving, 1 mL of 1 *M* NaOH was delivered into each vessel, exterior to the omnivial. A sterile 0.63-cm filter paper disc (Sensidiscs; BBL Microbiology Systems, Cockeysville, MD), impregnated with 10 μL of ^{14}C-labeled urea (2.0 GBq/mmol; Amersham Corp., Arlington Heights, IL) and diluted in water to 25 kBq/mL, was added to each omnivial. The final concentration of substrate was 0.25 m*M*, with a specific activity of 5.55×10^5 disintegrations per minute (DPM) per vial.

Treated tissue homogenates (1-mL aliquots) were added to the omnivials and the sealed reaction vessels were incubated at approximately 25°C for 1, 6, 12, 24, and 48 h. The reaction was terminated by injecting 0.2 mL of 1 *M* HCl through the septum cap into the omnivial. All incubations were performed in triplicate and positive controls, consisting of 0.3 mL of Jack Bean urease (0.33 units/mL; Sigma Chemical Co., St. Louis, MO) added to the homogenates via injection, were run for each of the treated homogenates.

Omnivials were removed from the reaction vessels 6 h after acidification to prevent spillage into the NaOH traps during transport to the laboratory. Each reaction vessel received 15 mL of aqueous counting solution (Amersham Corp., Arlington Heights, IL), and ^{14}C activity was measured using a liquid scintillation counter (Beckman LS 7500; Beckman Instruments, Inc., Palo Alto, CA). Counts were converted to DPM using an external standard to monitor quench. ^{14}C activity in the NaOH trap served as an indicator of ^{14}C-labeled urea mineralization.

TABLE 2. ^{14}C-labeled urea mineralization (mean $\log_{10}$ DPM in the NaOH trap after 48 h of incubation) in shark tissue homogenates treated with saline, O/129, and ampicillin. Means followed by the same letter are not significantly different at the 5% level as indicated by Tukey's Studentized range test.

Tissue	Urea mineralization in presence of: Saline	O/129	Ampicillin	SE
Blood	2.87 a	2.75 a	2.75 a	0.049
Liver	4.11 b	2.75 a	2.80 a	0.107

All DPM data were log transformed and analyzed as $\log_{10}$ DPM. Analysis of variance was performed using the Statistical Analysis System (SAS Institute Inc., Cary, NC) available on the University of Maryland IBM 4381 computer.

DPM at 48 h were compared among tissues and among treatments using analysis of variance and means were compared using Tukey's Studentized range test.

Results and Discussion

Because the muscle and kidney tissues could not be homogenized completely, the results were variable. Therefore, only those data for liver and blood homogenates were analyzed.

The liver homogenates showed a significant difference ($P < 0.05$) between control, i.e. saline, and antibiotic treatments (Table 2). Mean DPM for liver homogenates without antibiotic were 1.3 logs higher than those for samples to which antibiotic had been added, indicating a bacterial role in urea mineralization.

Blood homogenates showed no significant difference between control and antibiotic treatment, i.e. all treatments showed counts at background levels, indicating no urea hydrolysis occurring in the blood homogenates (Table 2). Comparison of data for blood and liver homogenate samples to which no antibiotic was added showed a significant difference ($P < 0.05$), with the liver DPM 1.3 logs greater than those recorded for the blood samples.

Liver homogenates treated with saline showed a marked increase in ^{14}C-labeled urea mineralization with time, reaching the same level for both sharks at the end of 48 h (Fig. 1a and 2a). No change in results of tests for mineralization by blood samples was noted (Fig. 1b and 2b).

Positive controls, i.e. tissue homogenates to which Jack Bean urease was added, showed no difference among treatments. Mean $\log_{10}$ DPM for the positive controls was 4.97 at 24 h (SEM = 0.066).

Observation of ureolytic activity in samples of healthy shark liver provides evidence, consistent with pure culture studies of ureolytic bacteria isolated from sharks, for a significant role of bacteria in shark urea homeostasis. The hypothesis that sharks carry an autochthonous bacterial flora is supported by results of extensive sampling of approximately 50 healthy, free-ranging sharks collected near the island of Bimini, Ba-

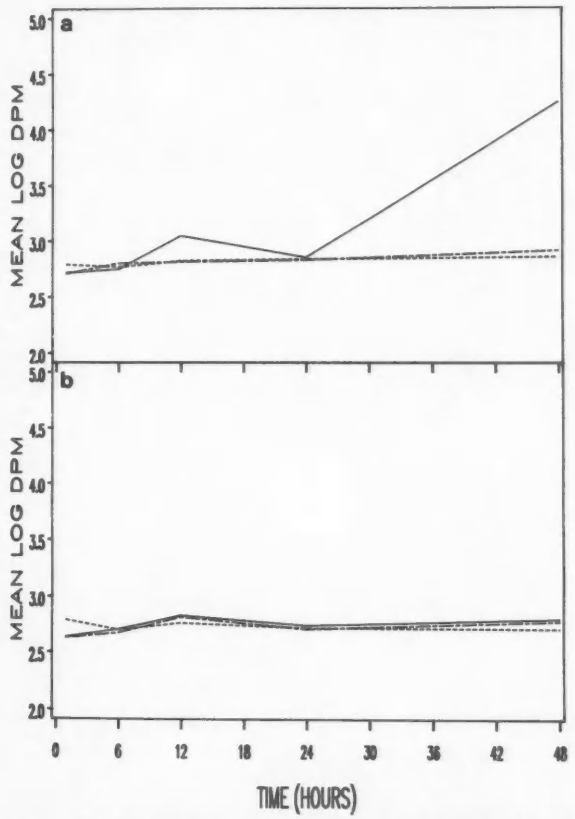

FIG. 1. Mean $\log_{10}$ DPM in NaOH vs. time of incubation of lemon shark (a) liver and (b) blood homogenates with ^{14}C-labeled urea. Homogenates were treated with saline (—), O/129 (---), and ampicillin (– - –).

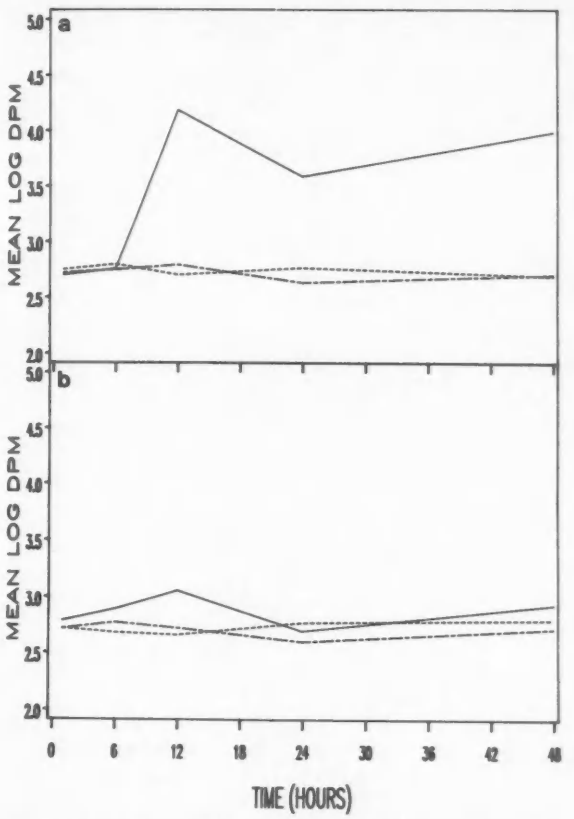

FIG. 2. Mean $\log_{10}$ DPM in NaOH vs. time of incubation of tiger shark (a) liver and (b) blood homogenates with ^{14}C-labeled urea. Homogenates were treated with saline (—), O/129 (---), and ampicillin (– - –).

hamas (Grimes et al. 1985). Typically, the blood of sharks is sterile, while other tissues, including muscle, kidney, liver, spleen, and the gastrointestinal tract, contain from 10^2 to 10^5 bacteria per gram of tissue (Grimes et al. 1985; and unpubl. data). Large numbers of bacteria are expected to be present in the gastrointestinal tract, but finding such populations in the internal organs of healthy animals is unusual. The majority of the bacteria isolated from sharks are able to hydrolyze urea and some have been shown to utilize CO_2 and NH_3, the products of urea hydrolysis, as sources of carbon and nitrogen (Grimes et al. 1984). Results of a numerical taxonomy analysis of 239 oxidase-positive strains of bacteria isolated from sharks showed several species of the genera *Vibrio* and *Aeromonas*, some of which are recognized human and marine animal pathogens (Grimes et al., unpubl. data).

That ureolytic bacteria are present and active in the liver but not in the circulatory system suggests that if healthy sharks are normally precolonized with ureolytic bacteria, stress during capture and maintenance in aquaria may compromise the immune system and result in a rapid systemic invasion. Since sharks, as all elasmobranchs, maintain a high plasma urea concentration (approximately 2%) as an osmoregulatory mechanism, systemic ureolytic bacteria could affect the animal very seriously by hydrolyzing urea, thereby increasing the blood pH. In pure culture, *Vibrio* spp. isolated from sharks are capable of elevating the pH of urea-containing media to pH 9.2 (Grimes et al. 1984).

Whether or not the autochthonous, ureolytic bacterial flora of sharks acts to control urea flux in tissues is a question that remains to be answered. While there is evidence of ureolytic bacteria affecting tissue urea levels, and thereby benefiting the host (Cheng and Costerton 1980), the existence of similar systems in shark tissue has not been investigated. Nevertheless, it is clear from results of this study that ureolytic bacteria can affect liver urea concentration by hydrolyzing and utilizing the urea of sharks.

Acknowledgements

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Fish in the Structure and Function of Freshwater Ecosystems: A "Top-down" View[1]

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The role of fish in regulation of the structure and function of freshwater ecosystems (lakes and streams) is examined with respect to effects resulting directly from their feeding activity, indirectly from size or other forms of prey selectivity, and from influences on nutrient cycling and transport. That fish have long had a legitimate place in limnological research is documented by a review of limnological books, texts, and journals. Recognition of their "top-down" effects, started largely from Czechoslovakian research (Hrbáček and others) in the mid-1950's, was popularized by American and German research (Brooks, Jacobs, and others) in the mid-1960's and has enjoyed a remarkable upsurge in the 1970's, continuing into the 1980's. Although size-selective predation has been an important phenomenon in stimulating widespread research on effects of fish on freshwater communities and their function, the importance of other mechanisms in such regulation is considered.

On examine le rôle du poisson dans la régulation de la structure et de la fonction des écosystèmes d'eau douce (lacs et cours d'eau), en particulier les incidences directes de leur activité trophique, l'incidence indirecte de la taille ou d'autres formes de sélection de proies et l'influence sur le cycle et le transport des bioéléments. Un examen de livres, de textes et de revues scientifiques sur la limnologie vient étayer la position légitime du poisson dans la recherche limnologique. Des chercheurs tchécoslovaques (Hrbáček et d'autres) ont été les premiers à reconnaître l'effet de haut en bas vers le milieu des années 1950. Cette notion a été popularisée par des chercheurs américains et allemands (Brooks, Jacobs et d'autres) vers le milieu des années 1960 et a fait l'objet d'une popularité renouvelée dans les années 1970 qui se poursuit dans les années 1980. Quoique la prédation selon la taille ait été un important phénomène pour ce qui est de la stimulation de recherches répandues sur l'incidence du poisson sur les communautés d'eau douce et leur fonction, on se penche sur l'importance d'autres mécanismes dans une telle régulation.

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Many ecologists by background, training, or persuasion have developed under a "bottom-up" viewpoint. We tend to envisage freshwater ecosystems as operating in a physical–chemical milieu which, largely through nutrient availability, conditions the trophic levels of the food web from primary producers leading to a terminus in some mix of predators that are often higher vertebrates. Indeed there were compelling arguments supporting such a viewpoint, as first clearly expounded by Lindeman (1942), and more recently reinforced by limnological studies undertaken for the International Biological Programme especially those following production and energy flow pathways (e.g. see Kajak and Hillbricht-Ilkowska 1972; Hall and Hyatt 1974). My purpose is not to suggest that we should abandon this perspective but rather that another, rising to prominence within the last decade or so, has provided some fresh insights and understanding. The idea that we might need a reversed viewpoint is not new. Straskraba (1965) wrote that "limnology generally accepts the classic scheme of influences: physical and chemical factors – phytoplankton – zooplankton – fish; very little attention has been paid to *reversed* influences" (my italics). But it is a view that I chose to adopt some time ago and now feel comfortable developing in tribute to Frank Rigler. It is an appropriate subject to dedicate to him because, as a limnologist, he was most concerned about the need for better communication between his science and that of fisheries biology (Rigler 1982).

Like so many buzzwords in ecology and limnology, "top-down" and "bottom-up" controls have crept into our recent literature and thinking with a variety of usages and implications. Some would include under the top-down category a number of processes affecting the whole trophic pyramid from the top level downwards (e.g. Andersson 1984). Others take a more restrictive view and regard top-down regulation as being mainly

[1]Frank Rigler Memorial Lecture, Society of Canadian Limnologists, January 1987, Richmond, B.C.

or even exclusively brought about by consumers (predators) and bottom-up regulation as being producer (food) affected (McQueen et al. 1986; Bowlby and Roff 1986). I prefer a more general definition, sharing Andersson's (1984) concern that, in our enthusiasm over the obvious importance of predation, we may underestimate or even ignore many other important biotic interactions. Furthermore, although I focus almost exclusively in this review on fish, I emphasize that they may be neither the only components near or at the top of the trophic pyramid nor the more functionally dominant ones. In some inland aquatic ecosystems, amphibians, reptiles, birds, or mammals may assume this role, while in others, invertebrate predators may be functionally paramount. Paraphrased in terms of fish, the top-down view includes those various ways in which fish affect the function and structure of an ecosystem in contrast with the bottom-up view which considers the ways in which food limitation and related physical–chemical factors affect fish, i.e. I will examine effects *of* fish rather than effects *on* fish.

Not surprisingly in such a rapidly developing area, several other publications relevant to the subject have come to my attention since the lecture was given in early January 1987. See especially Langeland and Larsson (1980), Lazzaro (1987), and several of the papers in Kerfoot and Sih (1987).

Limnological Legitimacy of Fish

Forel, long considered the father of limnology (Goldman and Horne 1983; see also Rodhe 1969; Elster 1974), founded the familiar physical–chemical–biological sequence of our science in the late nineteenth and early twentieth centuries and included some 56 pages (about 3.5%) on fish in his 1901 large "handbook" on limnology. Earlier, Louis Agassiz (1850) in the United States had published his book on physical and biological conditions in Lake Superior which included substantial treatment of its fishes. Of the two founders of the International Limnological Association in 1922, Thienemann and Naumann, the latter had much association with fish, working as he did out of the fishery laboratory at Aneboda, Sweden, and helping the local farmers in their attempts to develop inland commercial fish-farms. Despite the early inclusion of fish in limnological research and publication, that subject area seemed to decline at least in popular limnological texts from the 1930's to the 1960's. The 1935 first edition of Welch's text (Welch 1935) only makes passing reference to fish, as does that of Ruttner (1963). Even more recent texts of Reid (1961), Cole (1975), and Golterman (1975) give little or no attention to fish. But Wetzel (1975) changed this in his first edition and the limnology text of Goldman and Horne (1983) includes a whole chapter on fish and fisheries.

In the development of limnology in the United States, fish have received considerable attention (see relevant chapters in Frey 1963), and certainly this has been the case in eastern Canada (Smith 1963; Fry and Legendre 1963), the Laurentian Great Lakes (Beeton and Chandler 1963), and western Canada (see especially Rawson's papers listed in Northcote and Larkin 1963). However, virtually all of the work has been directed, until recently, by a very strong bottom-up rather than top-down viewpoint.

I will examine more quantitatively the limnological record to determine not only the extent to which fish studies are included, but also the contribution of top-down approaches to this body of limnological publication. The proceedings of the International Limnological Association (SIL) result from a congress every 3 yr at which limnologists from all over the world report their latest research. About 15% of the papers presented at these congresses, since the 13th in Finland in 1956, dealt in some way with fish (Fig. 1). A small fraction (5–10%, except for the Polish congress in 1965) of these fish papers up to the early 1970's concerned top-down viewpoints, i.e. fish effects. The 1974 congress in Canada and the two succeeding ones (Denmark and Japan) saw this contribution rise to about 20%. It has reached more than 30% at the recent congresses in France and New Zealand (Fig. 1).

Limnology and Oceanography (Fig. 2) has quite a different publication history of fish papers compared to that of SIL. Since its start in 1956 the contribution of fish papers fell rapidly to less than 5% by the late 1960's with a very slight rise since then. This pattern is not an unexpected one and follows long-standing and carefully enunciated objectives of the long-term editor (Edmondson 1984). On the other hand the proportion of those fish papers dealing with top-down effects rose dramatically in the late 1960's and has remained close to 100% thereafter (Fig. 2).

Ecology seems to have been a little more acceptive (if variably so) of limnological fish papers (Fig. 3), with high points of some 30% in the late 1950's and again most recently. Like that for *Limnology and Oceanography*, the contribution of top-down viewpoints to the fish papers rose sharply in the early 1970's to reach a high close to 80% in the 1980's.

The *Canadian Journal of Fisheries and Aquatic Sciences* (formerly *Journal of the Fisheries Research Board of Canada*) has seen a decline in the proportion of limnological papers dealing with fish (Fig. 4), dropping from a high of 80% in the mid-1960's to the recent low of about 30%. Again, after a couple of short-lived spurts in the 1960's, there was a sharp increase since the early 1970's in top-down papers dealing with fish effects.

This brief review provides evidence that fish do have a place in the limnological literature, although dependent on the journal, and that more importantly there has been a remarkable increase in interest since the early 1970's regarding top-down effects of fish in limnological publication.

Some Direct Effects of Fish on Limnological Variables

Water Transparency

Common carp (*Cyprinus carpio*) in their food searching activities at the sediment surface are often said to increase turbidity of ponds directly. Nevertheless there is conflicting evidence of such effects (Schäperclaus 1933; Hepher 1958; Utermöhl's comments in Hrbáček 1958; Threinen and Helm 1954; McCrimmon 1968; Fletcher et al. 1985). The bottom feeding behaviour described by ten Winkel and Davids (1985) for bream and by Sibbing et al. (1985) for common carp presumably would increase turbidity in the overlying water. I witnessed a marked reduction in water clarity (and macrophytes) of a small coastal lake (Hatzic) in British Columbia following introduction of common carp in the 1940's, but cannot be sure that their feeding activities were the only cause.

Fish such as the silver carp (*Hypophthalmichthys molitria*) which filter-feed directly on phytoplankton cells may improve water clarity, as the whole-lake manipulation experiments of Carruthers (1986) in Lake Orakai, New Zealand, showed so dramatically. However, the filter-feeding clupeid *Dorosoma*

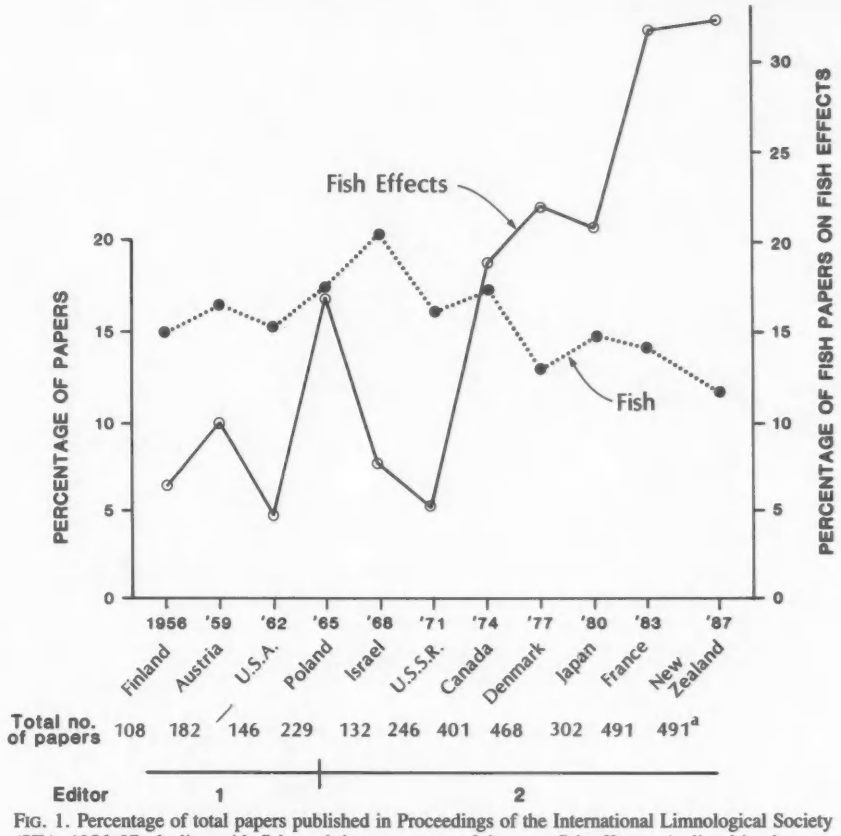

FIG. 1. Percentage of total papers published in Proceedings of the International Limnological Society (SIL), 1956–87, dealing with fish, and the percentage of these on fish effects. [a]As listed in abstracts presented orally at congress.

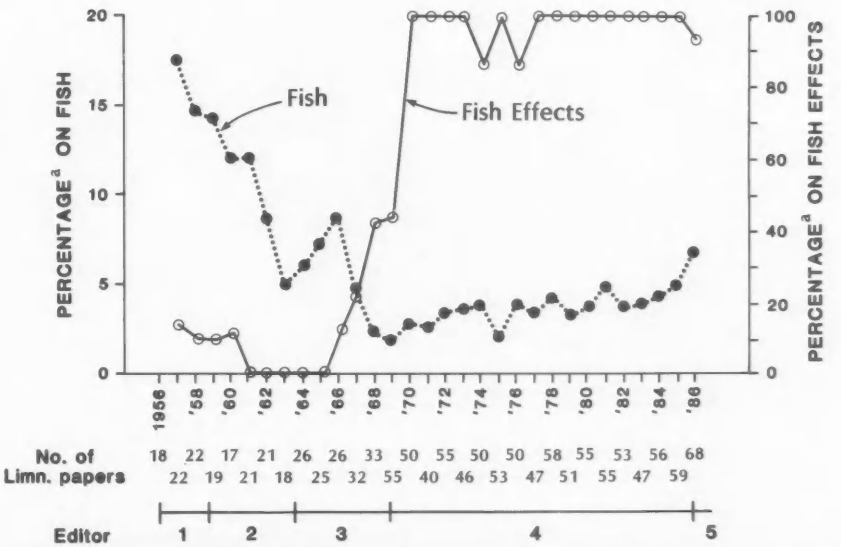

FIG. 2. Percentage of limnological papers published in *Limnology and Oceanography* dealing with fish, and the percentage of those on fish effects. [a]Three-year running average.

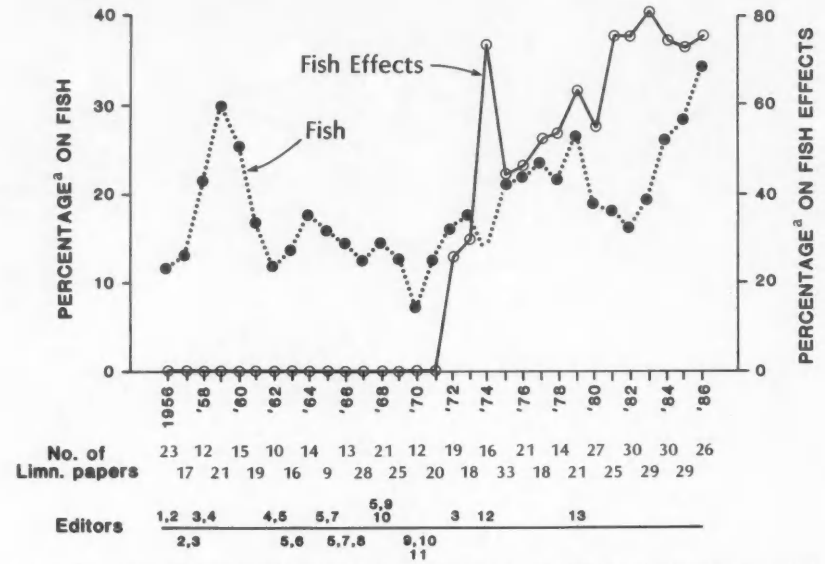

FIG. 3. Percentage of limnological papers published in *Ecology*, 1956–86, dealing with fish, and the percentage of these on fish effects. [a]Three-year running average.

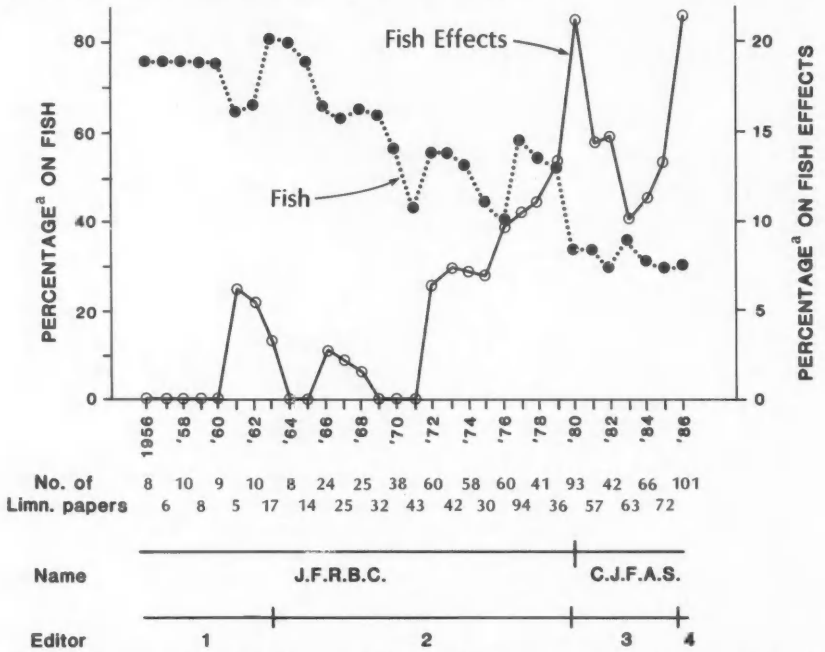

FIG. 4. Percentage of limnological papers published in the *Journal of the Fisheries Research Board of Canada* (later *Canadian Journal of Fisheries and Aquatic Sciences*), 1956–86, dealing with fish, and the percentage of these on fish effects. [a]Three-year running average.

cepedianum only filters the larger (>60μm) algae at a maximum rate, so its feeding activities mainly suppress large forms such as *Ceratium* (Drenner et al. 1984a) and actually enhance small forms with the result that turbidity increases and Secchi depths decrease (Drenner et al. 1986). On the other hand, in the experiments of Threlkeld (1987) with three different types of plankton-feeding fish (but no mortality) as well as with a fish mimic of zooplankton cropping, there were no increases in turbidity, suggesting that turbidity increase is more nutrient mediated than controlled by trophic-cascade effects.

Plant Biomass and Production

Phytoplankton

Many of the earlier reports on depression of phytoplankton

density by filter-feeding fish were largely based on circumstantial evidence (e.g. see Kutkuhn 1958; Prowse 1969). Cooper (1973) showed that a cyprinid (*Notropis spilopterus*) significantly reduced algal standing crop but increased net primary production. Two species of dorosomid shad may filter-feed on phytoplankton (20–60 μm size range) but, if anything, enhance rather than suppress densities of small phytoplankters (Holanov and Tash 1978; Drenner et al. 1984a; Drenner et al. 1986; Mummert and Drenner 1986), as does the omnivorous filter-feeding blue tilapia (*Tilapia aurea*) (Drenner et al. 1984b). However, in recent experiments with zooplankton feeding by *Dorosoma cepedianum*, where there were no mortalities and therefore no fertilization through regeneration of nutrients from decomposing fish, there was no enhancement of small phytoplankton density (Threlkeld 1987).

Although there is no doubt that silver carp in some cases can exert a strong control on phytoplankton biomass (Januszko 1974, 1978; Buck 1977; Carruthers 1986), in other cases, increasing the density of silver carp stocked along with common carp resulted in an increase in phytoplankton biomass as well as production in a series of Polish experimental ponds (Opuszynski 1980). Furthermore, a rather high fraction of the algal cells eaten by fish such as silver carp may be excreted without appreciable loss of chlorophyll according to Miura and Wang (1985), so that up to one third of the daily net phytoplankton production in Donghu Lake comes from algae already passed through fish.

Periphyton

Fish also may have major effects on periphyton biomass and production. The exclosure studies of Straskraba (1965) in Smyslov Pond showed that common carp may have a marked grazing effect on periphyton. In ponds without fish, periphyton abundance was severalfold higher than in those with fish (Spencer and King 1984). Pupfish (*Cyprinodon nevadensis*) in a desert stream fed heavily on algae and ingested about 17% of the annual primary production in the system (Naiman 1976). The algal-grazing minnow *Campostoma anomalum* had a strong depressant effect on periphyton in some pools of a prairie stream so that the substrate appeared barren (Power and Matthews 1983). Transfer experiments with algal-covered cobbles showed that these could be visibly grazed down within an hour and that a more than threefold reduction in periphytic biomass occurred within 24 h of grazing (22.0–6.3 mg ash-free dry weight·cm^{-2}). Other experiments with divided pools (minnows on one side, none on the other) clearly demonstrated strong grazing effects of fish on periphyton (Power et al. 1985). Periphyton grazing fishes include the cyprinid "chiselmouth" *Acrocheilus alutaceus* (Moodie and Lindsey 1972), the Japanese "ayu" fish *Plecoglossus altivelis*, and the pale chub *Zacco platypus* (Kawanabe 1959; Kawanabe et al. 1959) and some tropical freshwater fishes (Lowe-McConnell 1975).

Macrophytes

Common carp are often thought to strongly depress or even eliminate macrophytes in ponds either by direct grazing or by their burrowing activities on the bottom when feeding. Again there do not seem to be many clearly documented cases of such effects, but see Cahn (1929), Wolny (1962), and Straskraba (1965). Carp effected a 93% reduction in vegetative biomass and up to a 67% loss of total biomass in some species of macrophytes studied in an enclosure experiment (Macrae 1979). Removal of large numbers of white suckers (*Catostomus commersoni*) from an Ontario trout pond resulted in the reappearance of *Chara* on the bottom which apparently had been grazed down by their feeding activities (Ricker 1932). Likewise, Anderson (1950) reported increases in several different macrophytes after the removal of fish. Prowse (1969) discussed the possible use of two species of *Tilapia* as well as grass carp (*Ctenopharyngodon idellus*) for macrophyte control. The latter species can deplete macrophytes in small water bodies as shown by Terrell and Terrell (1975) where reductions in standing crops after 1 yr of grazing ranged between 79 and 89% in three ponds. Stocking of grass carp (44 fish·ha^{-1}) into a small (1.9 ha) New Zealand lake brought about a complete clearing of dense macrophyte growth (*Egeria densa* and *Eleocharis sphacelata*) within 2 yr without major changes in water quality (Mitchell 1980; Mitchell et al. 1984). Of the several recent reviews on effects of grass carp, Rowe and Schipper (1985) is probably most complete, although some interpretations of potential interactions with other fishes seem questionable.

Zooplankton Populations

Practitioners of pond culture in China and Europe must have recognized the effects of fish predation on zooplankton populations centuries if not millenia ago. Apparently, Steuer (1910), according to Straskraba (1965), was one of the earliest to record that zooplankton standing crops in fish ponds were affected not only by seasonal and weather changes but also by the density of the fish stocks.

As might be expected, many of the early studies documenting significant effects of fish predation on zooplankton populations came from studies on European common carp ponds (Gurzeda 1960; Wolny 1962; Hillbricht-Ilkowska 1962, 1963; Grygierek et al. 1966). Attention then turned to examining impacts of fish predation on zooplankton populations in larger water bodies where marked seasonality in its importance often was evident (Grygierek 1962; Gerking 1964; Pope et al. 1973; de Bernardi and Guissani 1975; Gliwicz and Prejs 1977; Jacobs 1977; Gliwicz et al. 1978; Beavers and Stavn 1979; Opuszynski 1980; Taylor and Gerking 1980; Gliwicz et al. 1981; Mills and Forney 1983; Grzybowska and Wodzinska 1984; Lehtovaara and Sarvala 1984; Neivrkla and Duncan 1984; Ripl 1984; Rognerud and Kjellberg 1984; Zaret 1984; Tessier 1986). In a Zambezi reservoir, fish imposed a short-term seasonality on zooplankters as a result of their high vulnerability to predation in near-surface waters at night during periods of full or nearly full moon (Gliwicz 1986).

Under severe fish predation, large declines in zooplankton standing stocks may occur. For example, high densities of zooplanktivorous alewife (*Alosa pseudoharengus*) and yellow perch (*Perca flavescens*) are thought to have depressed the 1984 inshore zooplankton abundance in Lake Michigan to 3% of its average level between 1975 and 1981 (Evans 1986). Nevertheless in moderately productive systems with high zooplankton abundance, strongly zooplanktivorous juvenile sockeye salmon (*Oncorhynchus nerka*) may crop less than 2% of the annual zooplankton production (Eggers 1978). Maximum fish (rainbow trout) predation in February on *Daphnia pulex* in Becker Lake accounted for only 6.8 and 24.6% of the production in 1976 and 1977, respectively, and usually was less than 6% (Taylor and Gerking 1980, 1985). Lane (1978) estimated that zooplankton losses from the major vertebrate predator in Gull Lake, the rainbow smelt (*Osmerus mordax*), were only 1–5% as important numerically as those resulting from invertebrate predation. Nevertheless there is no doubt that severe fish predation can eliminate some components of the zooplankton com-

munity in lakes. These will be discussed in detail later with respect to size-selective predation, but a few examples are useful here. Nilsson (1972) documented the elimination of the fairy shrimp *Polyartemia forcipata* from a large Lappish lake after introduction of arctic char (*Salvelinus alpinus*), and Gliwicz (1980) noted the extinction of planktonic cladocerans in alpine lakes after they were stocked with fish. Kettle and O'Brien (1978) reported earlier that arctic zooplankton was highly vulnerable to fish predation. Two species of planktonic chaoborid larvae were virtually eliminated from two British Columbia lakes in less than 2 yr after experimental fish introductions (Northcote et al. 1978). Fish predation, or lack of it, has been implicated in the absence or presence of several species of chaoborids in lakes (Pope et al. 1973; Northcote and Clarotto 1975; Stenson 1978; von Ende 1978, 1979) and in regional differences in zooplankton community structure (Nilsson and Pejler 1973). Differences in distribution, size, and burrowing characteristics of the larvae affect their availability to fish predation. Kokanee (*Oncorhynchus nerka*) predation is said to have been involved in the collapse of cladoceran populations in Lake Tahoe (Richards et al. 1975; Morgan et al. 1978; Goldman et al. 1979; Goldman 1981), but probably only in a minor way (S. T. Threlkeld, pers. comm.). Although *Mysis relicta* introductions were thought to be responsible for recent cladoceran declines in two Norwegian lakes, earlier decreases were in part the result of fish predation (Langeland 1981). Enclosure studies also document the rapid elimination of some cladocerans by fish (Andersson et al. 1978; Spencer and King 1984; Arcifa et al. 1986).

Experimental studies with manipulations and microcosms have indicated that fish predation can have variable effects on zooplankton populations. Kajak et al. (1972), using lake subdivisions, exclosures, and different fish stocking densities, showed increases in zooplankton biomass and production under high fish predation rates. In microcosm experiments, mosquitofish (*Gambusia affinis*) predation had strong depressant effects either on zooplankton abundance or on its timing (Hurlbert et al. 1972; Rees 1979).

Zoobenthos Populations

Some of the earliest experimental studies on the direct effect of fish predation on zoobenthic communities in lakes were those of Ball and Hayne (1952) followed by Hayne and Ball (1956) and later by Welch and Ball (1966). Fish removal and transfer were used to demonstrate that centrarchids could crop down zoobenthic invertebrate abundance but when so doing increased the rate of benthic production.

Paralleling the early European experimental studies of fish predation on zooplankton were a similar series examining impacts on zoobenthos. The two dominants in these studies were Lellák (1957, 1961, 1965, 1966, 1969, 1978) working in Czechoslovakia and Kajak (1958, 1963, 1964, 1972) and Kajak and Rybak (1966) working in Poland, although Hruška (1961, 1966) in Czechoslovakia as well as Assman (1962) in the USSR employed exclosure experiments in their attempts to quantify effects of fish feeding on benthic invertebrates. Others (Gurzeda 1960; Wojcik-Mizala 1965, 1966) likewise noted that zoobenthic abundance (mainly chironomids) decreased but production increased in ponds with higher densities of common carp. Lellák's experiments with exclosures or with carpless and carp-stocked ponds had indicated that in the absence of fish predation, zoobenthic abundance might be about double that in areas grazed by fish and that about 50% of the benthic population was consumed annually by carp. Although Kajak (1958) could find no significant difference in size of benthic populations in areas protected and unprotected from fish predation in backwaters of the Visla River in Poland, his later experiments in Lake Sniardwy demonstrated severalfold increases in chironomid larval densities in exclosures compared with those outside.

In the mid-1950's, Macan started his long-term study on the effects of brown trout (*Salmo trutta*) predation on macroinvertebrates of Hodsons Tarn wherein about a 5-yr period with no or very low fish predation was followed by a several-year period of relatively intense fish predation (Macan 1965, 1966a, 1966b, 1975). As Lellák and Kajak had found in their exclosure experiments with common carp, Macan showed that heavier fish predation reduced the standing crop of zoobenthos but increased benthic production, particularly for forms which were heavily fed upon such as the pulmonate snail *Limnaea pereger*. Two species of *Corixa*, widespread in the tarn before high trout density, were thereafter confined to thick plant cover where presumably the fish could not graze effectively. Other species such as *Notonecta obliqua* and several dytiscids were eliminated or virtually so by the trout. Berglund (1968) combined lake partitioning with exclosures and fish density manipulations to show the marked depressant effect on *Asellus* density of brown trout predation. Heavy perch predation was shown to remove up to 1.8% per day of the *Asellus* population of a small Finnish lake (Rask and Hiisivuori 1985).

From the early 1970's a series of studies, some using experimental manipulations, continued to describe consequences of fish predation on components of the zoobenthic community. Most of these demonstrated or suggested that fish predation can sharply reduce general zoobenthic abundance (Crowder and Cooper 1982; Ripl 1984), several groups of macroinvertebrates (Andersson et al. 1978; Jonasson 1981; Gelinsky 1984), particular groups or forms (insects (Martin and Fry 1972; Hurlbert et al. 1972), larval odonates (Morin 1984a,b; 1984b), hemipterans (Henrikson and Oscarson 1978), and chironomids (Walters and Vincent 1973; Kajak 1980; Hershey 1985; Pechlaner and Zaderer 1985)), or individual species (*Gammarus lacustris* (Koksvik and Aagaard 1984), crayfish (Biro 1977), and the pulmonate snail *Lymnaea elodes* (Brown and deVries 1985)). Some studies (Johnson 1977; Thorp and Bergey 1981a, 1981b), were unable to show any significant effect on zoobenthic density from fish benthic foraging.

Following earlier attempts to quantify the fraction of benthic production removed by fish predation, Gerking (1964) estimated that a 50% reduction in abundance occurred over the later part of summer once bluegill sunfish (*Lepomis macrochirus*) switched from heavy zooplanktivory to largely benthic foraging. In a marl lake studied by Momot (1967), brook trout (*Salvelinus fontinalis*) preyed on underyearling crayfish but utilized only 2.9% of the total net production and only 4.2% of the available net production of crayfish biomass in the lake. Even at high brook trout densities their predation was only capable of removing up to 40% of the crayfish production (Gowing and Momot 1979). Recent studies have shown that fish, especially if concentrated seasonally in the littoral zone, can crop a substantial portion of the benthic invertebrate biomass. Introduction of yellow perch into an Ontario lake resulted in a 60% decrease in total benthic biomass and a 50% reduction in mean invertebrate biomass in the littoral zone (Post and Cucin 1984). The littoral fish community of Lake Memphremagog forage

most heavily on benthic invertebrates, cropping annually 72% of the total dry weight available in the littoral zone even though this may not be a large fraction of food biomass present (Boisclair and Leggett 1985). Using enclosure experiments with yellow perch and pumpkinseed (*Lepomis gibbosus*) densities of 0.5, 1.0, and 2.0 times the total fish biomass in the littoral zone, Hanson and Leggett (1986) could not detect any significant cropping effects on total macroinvertebrate abundance or biomass, probably because the *real* availability of most benthic prey was much lower than indicated by standard sampling. In their study of the benthic energy budget for Mirror Lake, Strayer and Likens (1986) estimated that the total consumption of zoobenthos by vertebrates (fish and salamanders) was about 1 $g \cdot m^{-2} \cdot yr^{-1}$, with fish predation accounting for 15% of the benthic production.

Although studies of fish foraging on stream macroinvertebrates have been conducted for decades (e.g. see the classic study on the Horokiwi by Allen 1951), nearly all of these have been "bottom-up" in their orientation and only recently has there been a surge of "top-down" stream studies dealing with the effects of fish on benthic or drift invertebrates. One of the earliest was that of Straskraba (1965) who attributed the disappearance of *Rivulogammarus forsarum* in two tributaries of the Odra River to heavy grazing pressure exerted by dense brown trout populations which fed largely on this gammarid. Zelinka (1974), as noted in Macan (1977), manipulated trout and cottid abundance in two zones of a stream without detecting much change in ephemeropteran abundance in riffle areas. Similar results on abundance of riffle invertebrates have been reported in the extensive studies by Allan (1982, 1983) and Reice (1983) and also for most of the taxa in pools with and without trout (Hemphill and Cooper 1984). Nevertheless, one of my students in a manipulation experiment in a small stream showed a significant decrease in abundance but not production of riffle insects as a result of cutthroat trout predation (Griffiths 1981). In an experiment in forested and logged stream pools, Wilzbach et al. (1986) demonstrated a significant increase in drift density in pools from which trout were removed. Milstead and Threlkeld (1986) could not demonstrate any effect of darter (*Etheostoma spectabile*) predation on *Hyalella azteca* density, sex ratio, or fecundity in their experiments using pool enclosures which permitted in and out movement of prey, but they did find that the fish fed selectively on the larger amphipods causing a shift in the size structure of the population. Trout can affect the microdistribution of gerrids in pools, restricting them to the margins (Cooper 1984).

Healey (1984), in his review of fish predation on aquatic insects, provided a critical summary of the various techniques which have been used to manipulate fish populations in such studies (poisoning, netting, introductions, enclosures, exclosures) as well as the prey sampling methods. In some cases the effects of manipulatory and sampling techniques have been carefully evaluated in the interpretation of results, but that was not always the case. Healey noted that most of the studies were undertaken to describe consequences rather than to test specific hypotheses about predator–prey interactions. Although a valid criticism at the time, there appears to be an increase in hypothesis testing in more recent research in this field.

Other Consequences and Indirect Effects of Fish

Size-Selective Predation

Phytoplankton

Filter-feeding phytophagous fish must selectively remove a size (and probably shape) dependent fraction of algal cells passed through their filtration apparatus, but details of such selectivity are only well known for a few species. Threadfin shad (*Dorosoma petenense*) in their filter-feeding mode can retain small particles about 0.4 mm or less in size (Holanov and Tash 1978) although there seems to have been little work on how this affects the species mix of algae retained or on how it changes with fish size. More information on these questions is becoming available for the closely related and also filter-feeding gizzard shad (*Dorosoma cepedianum*) (Drenner et al. 1982a, 1984a, 1986; Mummert and Drenner 1986). The proportion of particles removed by the gill raker apparatus increases as a function of particle size, levelling off at 35 μm for fish in the 7–11 cm standard length (SL) range and at 55 μm for larger individuals (13–16 cm). Using selectivity index values computed from a filtering efficiency model, Mummert and Drenner (1986) estimated that gizzard shad of 5, 15, and 25 cm SL would selectively feed on particles >19, 40, and 63 μm, respectively, which could explain why phytoplankton becomes less important in their diet as they become larger. Blue tilapia (4–19 cm SL) feed selectively on particles >25 μm so that they may suppress larger forms such as *Uroglenopsis* and *Ceratium* while enhancing, perhaps by excretory fertilization, smaller forms such as *Rhodomonas*, *Chrysochromulina*, *Chlamydomonas*, and *Cyclotella* (Drenner et al. 1984b).

Zooplankton

In the 30 yr since the 1956 International Limnological Congress in Finland at which Hrbáček presented the first clear enunciation of the effects of fish size-selective predation on zooplankton and its consequences with respect to pond trophic status, there has been an upsurge of research in this area. Indeed O'Brien (1979) noted that "the dramatic impact that planktivorous fish may have on zooplankton communities has been a major focus of modern limnological research," and Zaret (1980a) used the subject as a central theme in his examination of the relative importance of competition and predation in determining the organization and structure of natural communities. There have been several other major reviews of the area (Hall et al. 1976; Nilsson 1978; Green 1983; Lazzaro 1987), as well as a recent symposium dealing in part with the subject (Kerfoot and Sih 1987). In my survey of only a portion of the relevant literature, I examined over 200 publications involving even more workers in this field. Interest in fish size-selective predation was slow in starting (Fig. 5) and for nearly a decade was centred on studies on Czechoslovakian river backwaters and carp ponds (Hrbáček 1958; Hrbáček and Hrbáčkova-Esslová 1960; Hrbáček et al. 1961; Hrbáček 1962; Straskraba 1965). The publication of Brooks (1965) and particularly Brooks and Dodson (1965) as well as Jacobs (1965) sparked more widespread interest in the subject, and from then until 1980, the number of papers published annually increased almost exponentially (Fig. 5). Publication rate in the area since 1980 has been fluctuating but still has averaged well over 10 papers annually.

Initially the research centred on "natural" experiments in which differences in fish and fishless (or nearly so) river backwaters were compared. Manipulative experiments quickly became the principal technique. Fish densities were controlled in ponds (Hrbáček et al. 1961; Hrbáček 1962), ponds were studied before and after fish removal by rotenone, and fish exclosures were used (Straskraba 1965). All of these studies showed an apparent size-selective feeding by fish inasmuch as the ponds

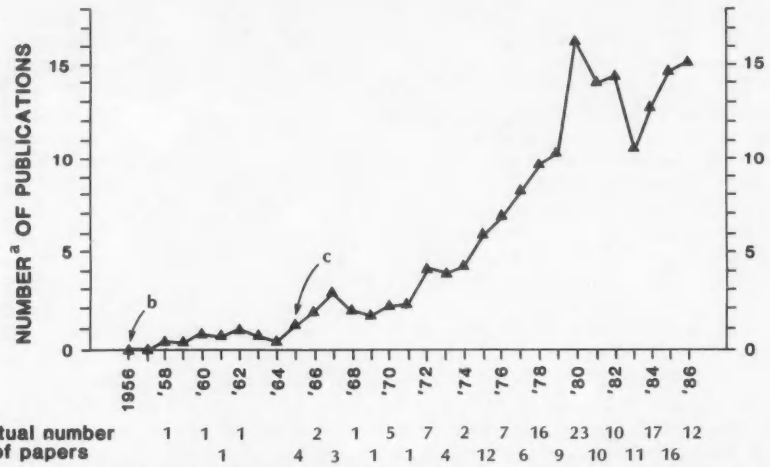

FIG. 5. Timing of the boom in publication on fish "size"-selective predation on zooplankton. [a]Three-year running average; [b]Hrbáček presents first clear outline of fish size-selective predation at 13th SIL Congress; [c]Brooks–Dodson paper in *Science (Washington, DC)*.

or areas without fish predation were dominated by cladocerans with large body size (*Daphnia longispina, D. pulex, Simocephalus vetulus*) whereas those exposed to fish were dominated by small cladocerans (*Bosmina longirostris, Scapholebris muconota, Ceriodaphnia* spp., *Daphnia cucullata*). The study of Brooks and Dodson (1965) also utilized a "natural" experiment to compare zooplankton community size structure before and after introduction of a highly zooplanktivorous fish (*Alosa pseudoharengus*) into a small lake. They showed a dramatic shift towards smaller body size in the presence of strong zooplanktivory by fish. Reif and Tappa (1966) reported similar effects of smelt (*Osmerus mordax*) introduction into a small lake. The major new contributions of these studies were the linkage of shifting zooplankton body size to grazing efficiency on phytoplankton and the extension of fish predation implications regarding lake community structure. The reviews of Nilsson (1967, 1978) also did much to place selective predation by fish into context with competition theory and interactive segregation of cohabiting species.

In the mid-1960's, differential fish predation on cyclomorphic forms of *Daphnia* was recognized (Brooks 1965; Jacobs 1965, 1966, 1967). Size-selective predation on *Daphnia* by rainbow trout and yellow perch was noted in North American lakes (Galbraith 1967) as well as for other species in an African lake (Green 1967). Interestingly the latter worker also found that it was the prey forms with large *eye* size that were removed selectively. This marks the beginning of the recognition that factors other than size alone were important in prey selection by fish, a viewpoint further extended to include movement, mobility, and other forms of behaviour as well as spatial distribution by Brooks (1968), Cramer and Marzolf (1970), and Hutchinson (1971). That there might be an important sex difference in prey predation rates was shown by Maly (1970) wherein the more active male *Diaptomus oregonensis*, even though smaller, suffered a higher predation rate than the females.

Hall et al. (1970) were some of the first to assess the relative importance of fish versus invertebrates as size-selective predators, and in their experimental pond systems the former had much greater influence on prey populations. Lane (1978) suggested the opposite for Gull Lake where smelt predation was only 1–5% as important numerically compared with invertebrate predation on zooplankton. Stenson (1976) found a sequentially shifting role for the two groups of predators and Lynch (1979) showed in an experimental pond that with fish (bluegill) as the vertebrate predator, small zooplankters increased whereas with *Chaoborus* as the main invertebrate predator, *Ceriodaphnia* was replaced by the large *Daphnia pulex*. Changes in body size of zooplankton in two lakes following experimental fish introductions seemed to result not only from direct size-selective predation by the fish but also by their rapid elimination of two chaoborid species, themselves invertebrate predators (Northcote et al. 1978). In Lake Washington the *Bosmina* morphs were thought to be more controlled by *Epischura* predation than by fish predation although the latter was still important (Black 1980). There is some evidence that severe fish predation on cladocerans may select for small clutch size and early maturation whereas that of invertebrate predation could have the reverse effects (Gliwicz 1981). Culver et al. (1984) showed that size-selective fish predation increased cladoceran reproductive output (per capita egg production); see also Dawidowicz and Pijanowska (1984) and Koksvik and Langeland (1987).

By the early 1970's, several workers had recognized that severe fish predation might decrease the abundance, biomass, and production of large zooplankton filtrators and not only could have the opposite effect on small filtrators, but might also increase their fertility (e.g. see Kajak et al. 1972; Archibald 1975). Nilsson (1972, 1978) provided a nice example to illustrate how fish selective predation can sequentially move down a size spectrum from large to smaller prey species. On the other hand, Wong and Ward (1972) showed that very young perch fry, because of mouth gape limitations, selectively preyed on *smaller* size classes of *Daphnia* until midsummer (see also Hansen and Wahl 1981). Later, when larger than 18 mm, they became selective on the larger sizes (Galbraith 1967). Also about this time, Zaret (1972a, 1972b, 1975) and Zaret and Kerfoot (1975) documented the importance of the size of the black compound eye in cladocerans in their selectivity by fish predators, although some argument exists concerning its significance (Confer et al. 1980; Kerfoot 1980; Zaret 1980b). Other

apparently visually obvious body parts such as eggs (both size and type) were also shown to be important in affecting prey predation rates (Kerfoot 1974; Mellors 1975; see also Jensen 1985; Hessen 1985). For transparent prey, such as *Chaoborus* larvae, whether or not they have recently had a full meal can greatly affect risk to fish predation (Giguere and Northcote 1987).

The mid-1970's witnessed a marked increase in the use of laboratory experiments, modelling, and behavioural observations to quantify fish predator–prey relationships (e.g. see Confer and Blades 1975; Confer et al. 1978; Kerfoot 1975; Hairston and Pastorok 1975; O'Brien et al. 1976; Eggers 1977; Gerritsen and Strickler 1977; Lynch 1977; Drenner et al. 1978; Jacobs 1978a, 1978b; Werner and Hall 1977, 1979).

Multiple lake comparisons, often providing a range in fish predation intensity, were well used in the 1970's to check the fit of the "Hrbáček pattern" regarding effects on body size and species composition of zooplankton communities (Stenson 1972, 1973; Nilsson and Pejler 1973; Northcote and Clarotto 1975; Pope and Carter 1975; Sprules 1975; Stavn 1975; Langeland 1978; von Ende 1978, 1979; Dodson 1979; Anderson 1980).

In the mid- to late 1970's, experimental limnologists began more intensive use of enclosures and exclosures to examine responses of zooplankton populations to selective fish predation. Some using this approach were Andersson et al. (1978) and Lynch and Shapiro (1981), but several others have followed recently (Arumugam and Geddes 1986; Cryer et al. 1986; Tessier 1986; Vanni 1986). Arcifa et al. (1986) demonstrated with enclosures the rapid response that the tropical zooplankton community could show to severe fish predation. Although the perch enclosure experiment of Raess and Maly (1986) did not demonstrate a significant effect on cladoceran size, there were significant shifts in depth distribution.

One of the more important developments in selective predation has been the demonstration that closely related species of fish, or a single species at different sizes (Mummert and Drenner 1986), or even at the same size at different times, may undertake two or more distinctive feeding methods, one of which may be size selective whereas the other is not. Janssen (1976a) showed that the alewife could feed on *Daphnia* in a highly size-selective manner but also could filter-feed in a non-size-selective mode, which according to his results from artificial feeders (Janssen 1976b) might be more effective. Cisco (*Coregonus pseudoharengus*) also have both selective and non-selective feeding methods (Janssen 1980), each of which singly or together can have different and spatially localized effects on a zooplankton community. Similarly, obligate and facultative planktivores may have differential impacts on zooplankton communities because of variations in their search or capture techniques (Janssen 1982), as well as size distribution, avoidance, or other characteristics of their prey. Studies by Drenner and McComas (1980), McComas and Drenner (1982), and Drenner et al. (1982a, 1982b) demonstrated the different effects on various components of the zooplankton community that may result from specialized forms of feeding by several atherinid species and by the gizzard shad. The latter is a filter-feeder and although not size selective, it feeds more heavily on prey with low escape ability, thereby shifting the zooplankton community towards more evasive prey types. Still more complex is the zooplanktivory of the cichilid *Sarotherodon galilaeum* in Lake Kinneret (Galilae) which shifts from an obligate particulate feeder to a facultative particulate and filter-feeder to eventually an obligate filter-feeder as it increases in size (Drenner et al. 1982b; Gophen et al. 1983), each of which can have differential effects on the zooplankton community. Roach (*Rutilus rutilus*) and bream (*Abramis brama*) have different prey strike abilities which make the latter far more effective at capturing copepods (Winfield et al. 1983).

Much of the discussion so far has focussed on cladocerans, but copepods also can be markedly affected by fish predation. In addition to changing life history characteristics such as diapause and annual or biennial fractioning in copepod populations (Nilssen 1977, 1980; Elgmork 1980; Gliwicz and Rowan 1984), selective predation by fish also may cause higher mortality in dark-coloured morphs (Luecke and O'Brien 1981; Byron 1982) as well as in ovigerous females and even alter their vertical distribution pattern (Hairston et al. 1983; Vuorinen et al. 1983; Winfield and Townsend 1983).

Since the early 1980's a number of workers have made attempts to predict selective predation outcomes of zooplanktivores, some based on a series of functional steps (prey location, pursuit, attack, retention) and with varying degrees of success (Lynch et al. 1981; Eggers 1982; Wright et al. 1983, Wright and O'Brien 1984; O'Brien et al. 1985; Wetterer and Bishop 1985; Bence and Murdoch 1986). In several cases the need is emphasized for revision in the importance which body size or even apparent size has been given in our previous thinking. Also there should be more realism built into the functional steps noted previously and more recognition that each must consider not only size-related changes in feeding mode but also more short-term shifts. A particulate zooplanktivore often may not feed in a single species patch of prey, so must be faced with alternative prey with different characteristics. In such cases its foraging efficiency may be significantly reduced (Persson 1985). Furthermore the feeding arena is never an ecological vacuum, so that competitive interactions with other zooplanktivores must be faced (Persson 1986), as well as that of predation risk for the zooplanktivore itself.

Zoobenthos

Recognition of the importance and implications of size-selective predation by fish on lake zoobenthos lagged at least a decade behind that in zooplankton communities. Hall et al. (1970), in their study of bluegill predation, noted that the effects on benthos were most clearly expressed by changes in body size distribution as the fish selectively removed the larger terminal aquatic stages of many benthic insects, a point evident also in Macan's studies (1965, 1966a, 1966b) of effects of brown trout predation on the benthos of Hodsons Tarn. Ware (1972) clearly showed in his experimental study that rainbow trout predation on epibenthic prey was sharply increased as prey size rose. Stein's work on both molluscs and crayfish (Stein et al. 1975; Stein and Magnuson 1976; Stein 1977) demonstrated not only that selective predation on benthos by fish occurs but that it affects prey behaviour differentially with size (small vulnerable crayfish alter their cover selection) and with sex (adult females have a strongly nocturnal activity pattern). They considered predatory fishes to be "keystone" species in that their predation is a key factor in structuring inshore benthic communities. That competitive interactions between cohabiting sunfish (Centrarchidae) species can markedly affect the size, type, and location of benthic prey utilization was nicely shown in the studies of Werner and Hall (1977, 1979) and Werner et al. (1977).

Laboratory experiments with common carp and goldfish (*Carassius auratus*) (Covich and Knezevic 1978) also have indicated that benthic predation can be size selective. Many field studies following on the earlier work of Cooper (1965), but often with experimental manipulations (exclosures, fish removals, or introductions), continue to provide evidence that predation in a wide range of benthophagous fishes is highly size selective (Kajak 1980; Crowder and Cooper 1982; Koksvik and Aagaard 1984; Morin 1984a; Post and Cucin 1984).

As in the research dealing with fish predation on zooplankton, so too in zoobenthos it soon was recognized that selectivity involved far more than merely prey size. Prey availability obviously could be affected by characteristics of the bottom such as sand or mud composition (Schutz and Northcote 1972; Lellák 1978) as well as presence and type of macrophyte cover (Covich and Knezevic 1978; Werner et al. 1983; Hershey 1985). Furthermore, prey behaviour including movement and activity patterns are important (Ware 1973; Macan 1975; Nilsson 1981; Pierce et al. 1985). Even reproductive behaviour such as amplexus in amphipods may expose paired prey to increased fish predation rates (Strong 1972, 1973). Particular feeding behaviours of fish may serve to enhance their predatory ability, as was shown long ago by Johannes and Larkin (1961). Rainbow trout were not as effective predators on amphipods and other benthic invertebrates in littoral *Chara* shoals as were the later introduced redside shiners (*Richardsonius balteatus*). Dolly Varden char appear to prey on mud-dwelling forms such as *Pisidium* by stirring up the surface sediment layer (Schutz and Northcote 1972) as do common carp (Kajak 1980) and bream, who in their disturbance of the bottom sediments in feeding also expose tube-dwelling chironomids to much higher invertebrate predation by water mites (ten Winkel and Davids 1985). As a final note on the biological complexities and interactions involved here, it should be pointed out that fish predators capable of both benthophagy and zooplanktivory may through the latter action enhance the rain of food particles onto bottom sediments, thereby increasing benthic productivity and also prey availability (Lellák 1978).

Although selective foraging on larger invertebrates has been a common theme emerging from benthic fish predation studies, there have been exceptions. For example, Stein et al. (1984) showed that redear sunfish (*Lepomis microlophus*) predation on three snail genera was related to shell strength and that size selection did not occur within any genus. Hershey (1985) presented evidence that small rather than larger chironomid larvae may be more vulnerable to fish predation because their near-surface tube dwelling makes them more available, a view also supported by the studies of Werner et al. (1983). Thus the comment of Stenson (1979) that much more work is needed before it can be said whether or not fish predation can have a correspondingly strong influence on structure and function within the benthic and "active fauna" in lakes as it does on zooplankton still seems valid.

Demonstration of size-selective predation in stream-dwelling fishes largely has developed within the last decade, notably by the studies of Bisson (1978), Allan (1978, 1981), Ringler (1979), Gilson and Benson (1979), Hemphill and Cooper (1984), Newman and Waters (1984), and Rose (1986). The mainly nocturnal drift of large forms may represent an effect of such predation on stream benthic invertebrates, a suggestion supported by the field and laboratory studies of Andersson et al. (1986). Bowlby and Roff (1986), in their study of 30 southern Ontario streams, claimed to find support for size-selective predation and "top-down" (predator limitation) hypotheses.

Fish

Two recent examples show that size selectivity, and no doubt other forms of selectivity, are involved in piscivory not only in higher fishes (Tonn and Paszkowski (1986) but also in lampreys (Cochran 1985). I will not attempt a review of other aspects of piscivory, but some relevant areas are covered by Gönczi and Nilsson (1984) and Nilsson (1985).

Zooplankton–Phytoplankton–Transparency Interactions

The first publications by Hrbáček dealing explicitly with fish predation as a major organizing factor in freshwater communities (Hrbáček 1958, 1962; Hrbáček et al. 1961), while considering the body size shift in zooplankton, also stressed the implication of this shift in terms of phytoplankton biomass and the associated changes in water transparency. Since then, many workers have discussed the correlations between zooplanktivorous fish density, algal density, and water transparency; some of the more recent include Węgleńska et al. (1979), Leah et al. (1980), Fott et al. (1980), Hurlbert and Mulla (1981), Elliott et al. (1983), Carpenter et al. (1985), and Scavia et al. (1986). Most recently, Carpenter and Kitchell (1987) have extended these interactions to include piscivory which they suggest may effect changes down through the food web to account for much of the remaining fraction (about half) of the variance in limnetic primary production that is not a result of nutrient inputs or physical processes. In some cases, phytoplankton biomass does not seem to be affected by fish presence or absence (e.g. see Munch et al. 1984).

Evidence of zooplankton–phytoplankton–transparency interaction has come from a series of experimental manipulations involving fish reductions, removals, or introductions (Stenson 1978; Henrikson et al. 1980; Benndorf et al. 1984; Shapiro and Wright 1984; Wright and Shapiro 1984), enclosures (Andersson et al. 1978; Tatrai et al. 1985; Arcifa et al. 1986), or tanks (Drenner et al. 1986). A recent study by Threlkeld (1987) is particularly useful in its evaluation of the two hypotheses that have been developed to explain the mechanism of algal enhancement by zooplanktivorous fish: (1) intense zooplanktivory by fish reduces zooplankton grazing pressure on phytoplankton and (2) zooplanktivorous fish increase nutrient cycling and thereby enhance phytoplankton. By clever experimental design and manipulation, the effects of planktivore predation and nutrient regeneration were simulated using a particulate feeding zooplanktivore (*Menidia beryllina*), an omnivorous pump filter-feeder (*Dorosoma cepedianum*), and a facultative omnivore (*Tilapia aurea*). Briefly summarized, there seems to be convincing evidence that (1) fish which do not die do not contribute to the enhancement of phytoplankton by the removal of zooplankton, (2) simulation of the fish feeding effect by removing zooplankton with a net does not result in phytoplankton enhancement, (3) addition of nutrients equivalent to measured nutrient excretion rates of fish enhances phytoplankton, and (4) addition of dead fish (even when zooplankton are not removed) enhances phytoplankton (Threlkeld 1987; and pers. comm.). These results suggest that we need to be much more cautious in interpretation of many of the experimental studies claiming to demonstrate phytoplankton increase via reduced zooplankton grazing when in fact the increase may well have been the result of nutrient excretion and/or regeneration.

The recent work by Vanderploeg et al. (1987) shows that year-to-year variation in calcite whitings in Lake Michigan probably are caused by predation-controlled variation in primary production. They attributed the absence of a whiting event in 1983 to increased salmon predation on zooplanktivorous alewife which in turn allowed populations of large *Daphnia* spp. to flourish and thereby graze down phytoplankton abundance, preventing intensive calcite precipitation through photosynthetic CO_2 uptake. Apparently this is the first example of a top-down control of the calcite whiting system.

Nutrient Cycling and Transport

As with nearly all top-down effects of fish in lentic freshwater systems, those linked with nutrient cycling again originate in large part from the observations of Hrbáček and his coworkers on Czechoslovakian river backwaters and ponds. In his 1958 paper, Hrbáček, responding to a question from Utermöhl, left no doubt that he has considered the role of fish in promoting nutrient exchange between the mud–water compartments of freshwater ecosystems and indicated that he already had work in progress to investigate this in detail. These results apparently were published much later (see Straskraba 1965) and showed that as fish density increased, so did the total amounts of dissolved nitrogen and phosphorus coming from greater exchange between the bottom sediments and overlying water because of fish searching activity for prey. Hepher (1958) noted that under the high pH levels in Israeli ponds, phosphorus added to the water was lost more quickly to the sediment when fish were present (probably through precipitation processes with $CaCO_3$) and that mixing of the mud–water interface by feeding common carp was the probable mechanism. Hilsenhoff (1965) noted that stirring up of the sediment by common carp as they feed affected both the chemistry and biology of overlying waters, although Straskraba (1965) could find no marked difference in ammonia nitrate concentrations between exclosures and open areas of carp ponds. In the microcosm experiments of Hurlbert et al. (1972), presence of a fish predator effected fundamental changes in phosphorus distribution in the system.

But it was Rigler himself in his plea for taking a more dynamic view of phosphorus pools, inputs, and interchanges in lakes that really launched this area of research into the orbit of modern limnology (Rigler 1973). Thus at the 19th SIL Congress, three papers were presented strongly linking fish with important processes in lake nutrient cycling. Fish were shown to be the major biomass compartment of the pelagic zone of eutrophic Lake Wingra (74% of the total), and in pond experiments, bluegill biomass represented 90% of the water column phosphorus (Kitchell et al. 1975). Although they thought that phosphorus remineralization by fish excretion was a minor pathway (see Lamarra 1975 for a contrasting view), two others were important: nutrient release from faeces and from fish decomposition. In one basin of a lake, Nakashima and Leggett (1980a) reported that fish carcass decomposition supplied up to 20% of internal phosphorus entering in spring. While phosphorus excretion levels might be at low (Nakashima and Leggett 1980a, 1980b) but relatively constant levels, they could act to stabilize planktonic cycles which otherwise could exhaust supplies of available phosphorus (Kitchell et al. 1975). In a series of enclosure experiments, Lamarra (1975) showed that common carp excretion was responsible for the major part of phosphorus loading and increase. Although Terrell (1975) could not find any significant changes in orthophosphates in ponds with or without grass carp at any season, much greater concentrations were evident in the pond sediments with fish, indicating rapid uptake from the water. Finally, Reinertsen et al. (1986) emphasized important differences in the form of nutrient release, that by fish being a heterogeneous patchy distribution which favours development of blue-green algae as opposed to the more homogeneous release by zooplankton which favours small edible algae.

In a series of recent studies following major reduction or complete removal of fish from lakes (Stenson et al. 1978; Henrikson et al. 1980; Smeltzer 1980; Shapiro et al. 1982; Shapiro and Wright 1984; Wright and Shapiro 1984), phosphorus concentrations decreased, and in one study where nitrogen levels were followed, they also decreased (Henrikson et al. 1980). Opuszynski (1980) reported a decrease in pond water nitrogen and phosphorus levels at increasing densities of silver carp stocking, presumably because phytoplankton biomass and production also had increased under the treatments. In enclosures with fish and in ponds with fish, nutrient levels (N or NH_3 and P) were shown to increase (Tatrai et al. 1985; Hambright et al. 1986).

Other experimental studies found that nutrients released by fish are in a highly available form for algal uptake, and in one lake, roach excretion and defecation could contribute between May and September about 30% of the total phosphorus supply to the epilimnion (Brabrand et al. 1985). Where algal growth might be iron limited, fish faeces could be an important source, and even fish epidermal mucous may make iron by chelation more available to blue-green algae (Barbrand and Faafeng 1984).

Size-selective zooplanktivory by fish represents an additional if indirect means of increasing nutrient release in locations readily available for uptake and recycling. In shifting the zooplankton community to smaller body sizes, fish planktivores can thereby increase phosphorus release rates (Bartell and Kitchell 1978; Kitchell et al. 1979; Kitchell 1980; Bartell 1981; Carlson and Schoenberg 1983) or cycling (Henry 1985).

As a further note on the involvement of fish in nutrient cycling, it should not be overlooked that they may play an important role in processing leaves of aquatic plants, especially in its more advanced stages (Hendricks et al. 1984).

Anadromous fishes have long been known to provide an important source of nutrients, especially to nutrient-poor inland waters (Gilbert and Rich 1927; Juday et al. 1932; Krokhin 1959, 1967, 1975; Donaldson 1967), and on years of peak sockeye salmon runs to an Alaskan river, phosphorus coming from salmon decomposition may exceed that from the drainage basin tributaries or from the atmosphere (Mathisen 1972). In his review of several sockeye salmon producing lakes, Stockner (1987) showed that phosphorus inputs from adult carcasses ranged from a low of 3% (Lake Washington) to a high of 58% of the total (Lake Ilianna) and that losses via outmigrant smolts were usually much less (1–20% of the total) except in a peak brood year for Iliamna when it reached 41.6%. Alewife influx into small streams can markedly affect their nutrient cycles and food chains, and although there is an eventual loss via juvenile emigration, the input in balance is much greater (Durbin et al. 1979). Fish spawning migrations from a lake into a stream may significantly increase its nutrient concentrations and promote greater heterotrophic activity as well as periphyton biomass and production (Richey et al. 1975). Large organic debris (logs, branches) in streams helps retention of fish carcasses so that nutrients from this source are not quickly flushed out in flood

TABLE 1. Summary of some top-down effects of fish on inland waters and their consequences.

Process	Limnological parameter affected	Mechanism and consequence
Direct feeding	Water transparency	(1) Food searching stirs up bottom sediments and lowers transparency
		(2) Intense phytophagous feeding may increase transparency; reverse effects also demonstrated, depending on algal sizes grazed and extent of fertilization effects
	Nutrient release, cycling	(1) Benthic food searching increases mud–water nutrient exchange
		(2) Littoral vegetation grazing, processing increases nutrient cycling
	Phytoplankton	(1) As in (2), water transparency
		(2) Heavy grazing commonly increases production
	Periphyton	(1) Strong cropping effect on biomass shown for lakes and streams
	Macrophytes	(2) As for periphyton
	Zooplankton	(1) Strong cropping effect on abundance especially of larger forms
		(2) Some evidence of increased production
	Zoobenthos	(1) Strong cropping effect on abundance common but not invariable in lakes and streams
		(2) Distribution and size of fish feeding cause marked seasonality in effects
		(3) Production often increased in lakes but not so in streams
Selective predation (size, visibility, motility)	Phytoplankton	(1) Shifts in relative abundance of algal size and species composition
	Zooplankton	(1) Shifts in relative abundance of species reduces algal grazing efficiency and water transparency
		(2) Changes in clutch size and maturation timing
	Zoobenthos	(1) Heaviest predation on large body-size forms affects their cover selection, activity patterns, and reproductive behaviour (both lakes and streams)
	Nutrient release	(1) Shift to smaller body size (zooplankton) increases nutrient release
Excretion	Nutrient release	(1) Liquid release provides quick, patchy availability
		(2) Faeces release provides slower patchy availability after remineralization
		(3) Epidermal mucous release increases Fe availability to algae via chelation
Decomposition	Nutrient release	(1) Carcass remineralization provides slow, patchy releases
Migration with excretion/ decomposition	Nutrient enrichment	(1) Transport of excreta or body decomposition products from high nutrient to low nutrient regions (sea to inland waters, stream lower to upper reaches, lake layers)

flows (Cederholm and Peterson 1985). Even though amounts of phosphorus transported by fish migration into headwaters of a stream were only about half that lost by leaf discharge and less than 0.1% of that lost in stream outflow, they were still considered important in maintaining phosphorus reserves in those reaches (Hall 1972). On the other hand, catadromous fish migrations could result in appreciable nutrient losses from small lakes, judging from data presented by Smith (1966).

Finally it should be noted that diel vertical migration by fish may be an important means of transporting nutrients from deeper waters, perhaps below a thermocline, up into surface pelagic or littoral waters. Peamouth chub (*Mylocheilus caurinus*), which feed actively on zoobenthos at depths of 20 m or more in a lake and undergo regular nocturnal vertical migration to the surface during summer and early autumn (Northcote et al. 1964), could be a significant means of nutrient replenishment in upper or onshore regions.

Eutrophication

Although the effects of fish predation and other interactions relevant to lake eutrophication have been alluded to already, it would be useful to draw these together with some additional comment. The subject was reviewed earlier (see Brooks 1969; Larkin and Northcote 1969) but has developed considerably in the recent decade, notably by the work of Shapiro and his associates as well as by Langeland and others (see especially Langeland and Larsson 1980; Langeland 1982; Reinersten et al. 1986). The cyclic feedback effects resulting from fish size-selective predation surely enhance eutrophication processes as do many of their other effects (nutrient cycling, for example). But changes in fish species often have been viewed as a consequence of eutrophication rather than a cause.

Conclusion

I believe there is convincing evidence that, while predation is a major means of top-down control by fish in the structure and function of freshwater ecosystems, there are other processes of importance involved that need investigation. Many of the processes and their interactions discussed in this review are summarized in Table 1; reference to fish species and study

authors may be found in the appropriate section of the text. Foraging activities of fish can directly affect water transparency and thereby indirectly influence primary as well as secondary production. Fish feeding has direct effects on phytoplankton, periphyton, and macrophyte communities in addition to those usually studied in plankton and benthic communities or on stream drift and benthos. Fish size-selective predation is not restricted to zooplankton but includes algae and drift and benthic invertebrates in streams as well as lakes. The complex interactions between zooplankton, phytoplankton, and water transparency that have been revealed by recent studies on size-selective zooplanktivory by fish probably have parallels in other prey groups. In addition, fish can have major influences on nutrient cycling and transport. These may involve long distance exchanges between marine and inland waters as well as less extensive but still important transfers between lakes and streams, between lower and upper reaches of streams, or between lower and upper layers of lakes.

In all of these means of fish involvement in top-down processes, there is an amazing degree of complexity. Predation must be considered in relation to competition, as well as risk. Prey usually are selected from a varied menu in which size is but one of many factors. The menu itself changes seasonally and even daily and with changing size and other aspects of the predator. These are but a few of the more obvious realities evident in the complexity of fish involvement in biotic interactions.

In view of this complexity, it may be naive and simplistic to expect the structure and function of freshwater ecosystems to be "controlled" by mainly top-down or bottom-up processes. The latter can have many important effects on the former and vice versa, as I have indicated throughout this review. Both must be involved, although in some systems or at some times, one or the other may be paramount. Undoubtedly, solar energy and nutrient inputs and dynamics of an ecosystem set its overall level of production, so to that extent the control may be envisaged as bottom-up, but within those limits, some of the "coarse-tuning" and much of the "fine-tuning" of structure and function in the system results from the complexity of top-down processes as discussed.

But our technical, experimental, and computational capabilities for dealing with such complexity have enlarged greatly over the last 30 yr. Even more importantly, we are passing from a perhaps necessary emphasis on describing consequences in our experimental approaches to testing specific and important hypotheses dealing with fish and their effects on freshwater ecosystems. If nothing else stands out in this review of recent fish–limnological literature, it is the marked increase in specific hypothesis testing that has occurred within the last 5–10 yr. And it is for young limnologists to continue this movement, while not neglecting the excitement of observing or the value of describing their favourite organism, interaction, or process. To effectively combine the careful observing with the posing and testing of good hypotheses will be a challenge, but one which would surely have been promoted and enjoyed by Frank Rigler himself.

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LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

Comment on "Nutrient Limitation of Phytoplankton Growth in Brackish Coastal Ponds" by Caraco, Tamse, Boutros, and Valiela (1987)

An ironic omission in a recently published paper (Caraco et al. 1987) apparently resulted from the authors being unaware of a paper (D'Elia et al. 1986) being published in the same journal at about the time as the original submission of their manuscript. Thus they say that "...there is relatively little data on nutrient limitation of brackish estuaries..." and, more importantly, do not take into account any possible seasonal effect on the nutrient limitation – salinity relationship described by D'Elia et al. (1986).

Results of D'Elia et al. (1986) for a section of the Patuxent estuary where salinity varied between 3.2 and 10.5 (precise salinity data from Sanders et al. 1987) over the course of 13 mo (1983–84) indicate that phytoplankton were limited by phosphorus (P) in the fall and winter (October–February; salinity 6.0–10.5) and by nitrogen (N) in the late spring and summer (June–September; salinity 4.2–10.5). Limitation indexes are calculated from the data of D'Elia et al. (1986) by dividing in vivo chlorophyll for N and for P enrichments by that of the control for each day of the experiment and then averaging the time series of data (Fig. 1A) in a fashion similar to that of Caraco et al. who used maximal index values.

One of the objectives of the Caraco et al. paper was "to determine if there was a switch from P to N limitation along a salinity gradient." They interpreted their combined results (i.e. July and October (Caraco et al., fig. 2)) as showing P limitation at salinities from 0 to 6.5 (but not at 6.8) and N limitation at 31 and 32 but not at 30. Presumably the supply of N and P was balanced from salinities of 6.8–30 because a response was noted with combined N and P but not with single element additions. In light of the seasonal response information of D'Elia et al. (1986), it would be safer to indicate only that in July the switch from N to P limitation occurred between 3.5 and 31 (i.e. N limitation in July at 31 and above and P limitation occurred at 3.5). In October there is no evidence of N limitation and of P limitation at 6.5 and below.

The experimental design of D'Elia et al. (1986) was replicated at the Virginia Institute of Marine Science (VIMS; Webb et al., unpublished) from 1985 to 1987. These experiments were carried out in the lower York River estuary where the salinity varied from 12 to 24.5 during an 18-mo period. The experiments differed from those of D'Elia et al. (1986) in using smaller microcosm vessels (50 L), running the experiments for a shorter period (10–16 d), and using extracted rather than in vivo chlorophyll measurements. The York River results for the seasonal pattern of nutrient limitation index (Fig. 1B) parallel those from the Patuxent. Using these combined data sets and the assumed end member conditions that freshwater phytoplankton are always P limited and oceanic phytoplankton are always N limited, I provide the following scenario. Phosphorus limitation displaces N limitation down the estuary (i.e. Chesapeake Bay tributaries) during the fall and winter to salinities as high as 25 (York River, February 1987) and in turn is displaced by N limitation up the estuary in summer to salinities below 3.8 (Patuxent River, May 1984). I interpret the mechanism of this change of position of the P to N limitation switch as related to variations of the relative inputs of N and P to the estuary. The seasonal distribution of dissolved phosphate in estuaries shows a cycle, with the maximum concentrations in summer and the minimum in winter (Taft and Taylor 1976; D'Elia et al. 1986) in contrast with coastal waters where the reverse is true (Strickland and Austin 1960). Inputs from point sources such as sewage can be expected to be relatively invariant seasonally. A large input of non-point source nitrate from wintertime runoff finishes the scenario of wintertime phosphate limitation and summertime N limitation in estuaries — Kenneth L. Webb, *Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062, USA.* (J9289)

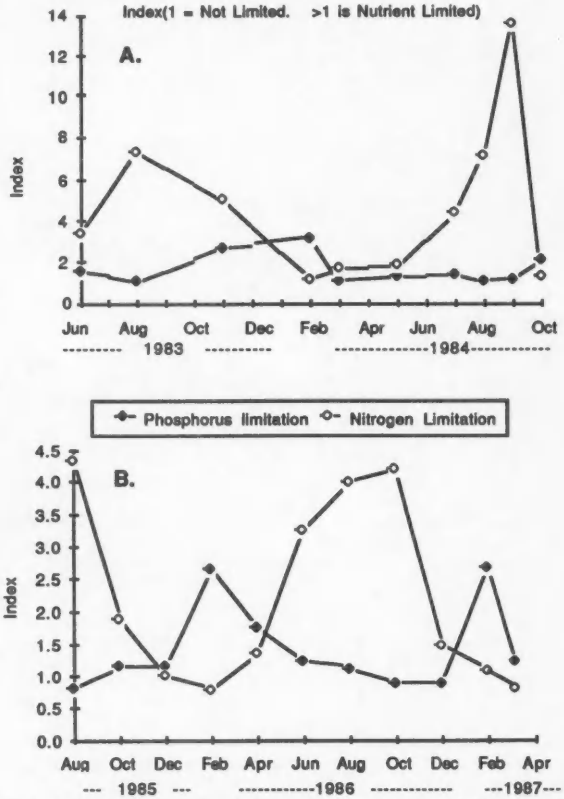

FIG. 1. Nutrient limiation indexes (treatment chlorophyll : control chlorophyll for the (A) Patuxent River experiment and (B) York River experiment.

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What Is the Mechanism Behind the Seasonal Switch between N and P Limitation in Estuaries?

There is a relatively clear understanding of nutrient limitation in freshwater lakes (Vollenweider 1968; Schindler 1977). There is, however, presently no clear understanding of nutrient limitation in brackish estuaries or coastal ponds (Boynton et al. 1980; Schindler 1981; Graneli et al. 1986). Thus, in our recent paper (Caraco et al. 1987), we decided to address a few aspects of nutrient limitation in brackish systems. The ponds we studied varied in salinity between 0 and 31, but were all within 2 mi (3.22 km) of each other and had similar geological and hydrological settings. Additionally, the nutrient budgets of all these systems are dominated by groundwater inputs and are likely, therefore, to have similarly high N:P loading ratios. The conclusions of our study were that (1) planktonic primary production in these brackish ponds was nutrient limited and N and P were important limiting nutrients and (2) P was relatively more important in limiting phytoplankton growth than was N in the less saline coastal ponds; in the more saline coastal ponds, N became as, or more, important than P in limiting phytoplankton growth. Based on our results, and of data in the literature, we suggested that N versus P limitation might be a salinity-related phenomenon. The salinity where a switch from P to N limitation occurs, however, is likely to be controlled by external N:P loading ratios.

In our paper, we neglected to cite a recent paper of D'Elia et al. (1986) which discussed seasonal patterns of nutrient limitation in the Patuxent River estuary. We regret that we were unaware of this paper. As Webb has written a comment on this ommision, he obviously feels that, had we been aware of the work of D'Elia et al. (1986), our paper would have been changed in some major way(s). First, Webb feels that our statement that "...there is relatively little data on nutrient limitation of brackish estuaries or coastal ponds" was unwarranted. However, the addition of just one more paper on nutrient limitation of brackish systems hardly changes this picture and, as of yet, no clear generalization can be drawn about the importance of N versus P limitation or the factors controlling N versus P limitation in estuaries or brackish ponds.

More importantly, however, Webb feels that we should have examined the seasonal aspects of N versus P limitation as was the focus of the study of D'Elia et al. (1986) for the Patuxent and Webb et al. (unpubl.) for the York River estuary. Webb states that in estuaries, seasonal changes in N:P loading can occur and cause a given system to switch seasonally from P to N limitation. For the coastal ponds we studied, there is no evidence that there is a seasonality in N:P loading and, thus, no mechanism for a seasonal switch from P to N limitation. Additionally, even if such a seasonal switch were occurring, it would not have affected our conclusions (above). Lastly, I would like to discuss the possibility that seasonal salinity changes, and not loading changes alone, actually caused the seasonal switch from N to P limitation discussed by Webb in the Patuxent estuary (D'Elia et al. 1986; Sanders et al. 1987).

Our study on coastal ponds was not set up to look at seasonality of nutrient limitation, and the only seasonal aspect to our data was that our study was done in two parts: (1) a "pilot" study using water from two brackish ponds (S = 3.5 and 31) and nearby seawater (S = 32) which was done in the summer and (2) a more complete study using water from five ponds in the fall (S = 0–30). (In our summer pilot study, we found that the coastal pond of lower salinity (3.5) was P limited, while the more saline coastal pond (31) and coastal seawater were N limited. In the fall study, the three freshest ponds showed P limitation, while the two more saline systems showed joint N and P limitation (i.e. phytoplankton growth increased when N and P were added in combination but not when N or P was added alone). Thus, either set of data, taken individually, would agree with the notion that P versus N limitation is a salinity-related phenomenon. Further, because we actually concluded that changes in N:P loading ratios could effect the salinity at which a system switched from P to N limitation, a seasonal change, driven by changes in N:P loading, would have been consistent with our conclusions. However, there is no clear indication, for these coastal ponds, that seasonality in N:P loading ratios is a critical variable in causing a shift from N to P limitation. While it is true that the only clear incidence of N limitation was found in the summer, the only two systems which showed N limitation, in fact, had the highest salinities. Lastly, in the brackish ponds we studied, there is no evidence that N:P loading ratios change seasonally, as groundwater is the major

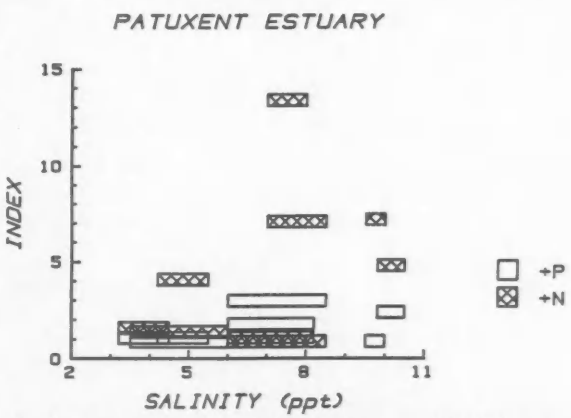

FIG. 1. Nutrient stimuation of phytoplankton growth (index) versus salinity range during nutrient addition experiments in the Patuxent estuary. Salinity data are those presented by Sanders et al. (1987), while nutrient stimulation data are those seasonal data presented by Webb (comment preceding). The June 1983 data point, presented in Webb's fig. 1, is ommited from our analysis, as Sanders et al. (1987) presented no salinity data for this addition experiment. The nutrient stimulation index (described by Webb) is equal to the mean chlorophyll concentration in enrichments (N or P) over that in the controls.

nutrient input. Thus, although it is certainly a possibility that seasonal N:P loading ratios cause shifts from N to P limitation in many brackish systems, our data neither support nor disprove this idea. Further, as mentioned above, even with this possible seasonal shift, our data support the idea that salinity, perhaps through salinity-related changes in N or P cycling (Howarth and Cole 1985; Fox et al. 1986), tends to influence if a system is N or P limited.

Although we have no evidence that there is strong seasonality in N:P loading ratios in the systems we studied, in some brackish systems it is known that there is seasonality in external N:P loading ratios which may influence seasonality of nutrient limitation (Graneli et al. 1986). Webb states, although he presents no data to support this, that there is a strong seasonality in N:P loading ratios in the Patuxent estuary. He believes that this seasonality of N:P loading, and not salinity, is the key variable determining seasonal switch from N to P limitation. In fact, Webb completely dismisses the possibility that salinity plays a role in determining N versus P limitation in the Patuxent estuary. He states "...phytoplankton were limited by phosphorus (P) in the fall and winter (October–February; salinity 6.0–10.5) and by nitrogen (N) in the late spring and summer (June–September; salinity 4.2–10.5)." Thus, Webb is suggesting that for the Patuxent there is clearly no trend of N limitation at higher salinities and P limitation at low salinities. However, a closer examination of the salinity data for the same Patuxent experiments (Sanders et al. 1987) shows that salinity is a reasonably good predictor of N versus P limitation (Fig. 1). If one were to look at the phytoplankton response to N or P enrichment in the Patuxent under high salinity (above 7 for the entire enrichment period) versus low salinity (falling below 7 during some or all of the 15- to 30-d enrichment), the trend toward N limitation at higher salinities would be evident (Fig. 1). Indeed, the average N stimulation of phytoplankton for the low-salinity period was less than 30% of that in the high-salinity period (2.1 as compared with 8.4 times the control, no enrichment treatment). In contrast, the average P stimulation of phytoplankton for the low-salinity period was 20% higher than for the high-salinity period. This same type of salinity analysis would be interesting for the York River data; unfortunately, Webb does not provide the salinity data needed for this analysis. However, at least for the Patuxent estuary, there is some evidence that seasonality of salinity as well as seasonality of N:P loading ratios could cause the observed shifts from N to P limitation.

I would like to provide one last piece of evidence from studies on the Baltic that some salinity-related change in nutrient cycling, and not just shifts in loading ratios, may be critical in determining N versus P limitation. The Baltic Sea is divided into several sub-basins of varying salinity; data indicate that the external N:P loading ratios to these sub-basins show no clear relationship to salinity of the sub-basin. External N:P loading ratios to the Bothnian Bay, Bothnian Sea, and Baltic proper (in order of increasing salinity) are 40, 46, and 31, respectively (Larsson et al. 1985; N_2-fixation excluded this as an internally controlled process). Surface water N:P ratios do not directly reflect N:P loading ratios, however; in the Bothnian Bay, Bothnian Sea, and Baltic proper, surface water inorganic N:P values are roughly 70, 16, and 8, respectively (Fonselius 1978). Comparing loading ratios with surface water ratios, it becomes evident that some internal process has altered the N:P ratios in these systems (denitrification, N-fixation, P sorption/desorption, etc.) (Nixon 1981; Cole et al. 1986; Fox et al. 1986). Further, the direction of this alteration (increasing or decreasing N:P ratios with respect to loading) appears to be related to the salinity of the system. In the Baltic Sea, therefore, while loading ratios to all three sub-basins would indicate that the basins should be P limited (N:P loading ratios greater than 16), P limitation occurs, in general, only in the least saline, Bothnian Bay (Alasaarela 1979; Graneli et al. 1986). Thus, while the importance of external loading versus internal cycling processes has not been completely worked out, it is clear that the internal cycling can play a role in determining if brackish systems are N or P limited — Nina Caraco, *Institute of Ecosystem Studies, Cary Arboretum, New York Botanical Garden, Box AB, Millbrook, NY 12545, USA.* (J9443)

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 44, No. 3 — article by D. W. Welch: *Frequency domain filtering of age-structured population data.*

Page 617: Equation A8 should read as follows:

$$A_{\text{Lim}} = \hat{\bar{a}} - \frac{\delta_b \text{Cov}(ab) \pm \sqrt{\left\{\delta_b^2 - \frac{p^{(M-1)}}{M(M-p)} F(p,M-p,0.05)\,\text{Var}(b)\right\}\left\{\text{Cov}(ab)^2 - \text{Var}(a)\,\text{Var}(b)\right\}}}{\text{Var}(b)}.$$

VOLUME 44, No. 4 — article by C. C. Parrish: *Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection.*

Page 724: The caption to Table 1 should read as follows: "Compounds representing aquatic lipid classes that can be resolved in a three-step separation. Representatives in the second compound column cannot be completely separated from those in the same class in the first column, but can be completely resolved from representatives in other classes using the solvent systems described in the text."

VOLUME 44, No. 4 — article by R. Knowles and D. R. S. Lean: *Nitrification: a significant cause of oxygen depletion under winter ice.*

Page 743: Line 7 of the résumé should read "était de deux à quatre ordres de grandeur trop faible..."

Page 745: Last entry in the first column in Table 2 should read "$\frac{\text{BOD}_N}{\Delta O_2}$ %".

Page 746: Line 3 of the caption to Fig. 3 should read "presence of acetylene (●) or nitrapyrin (Δ)..."

VOLUME 44, No. 4 — article by C. F. Wehrhahn and R. Powell: *Electrophoretic variation, regional differences, and gene flow in the coho salmon* (Oncorhynchus kisutch) *of southern British Columbia.*

Page 824: Equation 4 should read as follows:

$$(4)\quad \ln L(M) = r \ln C(M) + (4M\bar{x} - 1)\sum_{i=1}^{r} \ln x_i + (4M[1-\bar{x}] - 1)\sum_{i=1}^{r} \ln (1-x_i) - r \ln 2N + (R-r) \ln f(0).$$

Page 827, right column: Lines 17 and 18 should read "...The Oregon fish have less than 1/15 of the predicted variance...."

VOLUME 44, Suppl. 1 — article by J. A. Shearer, E. J. Fee, E. R. DeBruyn, and D. R. DeClercq: *Phytoplankton productivity changes in a small, double-basin lake in response to termination of experimental fertilization.*

Page 48: The second sentence of the second paragraph of the Methods section should read: "Annual addition rates were 1.81 $g \cdot m^{-2}$ for N and 3.46 $g \cdot m^{-2}$ for C in both basins, and 0.34 $g \cdot m^{-2}$ for P in the NE basin."

CAN. BULL. FISH. AQUAT. SCI. 215 — chapter by M. C. Healey and R. R. Wallace: *Canadian aquatic resources: an introduction.*

The correct sequence for pages 6, 7, 8, 9 is 8, 9, 6, 7.

ANNOUNCEMENTS/AVIS

Functional Development of Sensory Systems and Acquisition of Behavior in Larval Fish

Announcement and Call For Papers

A special session of the 12th Annual Larval Fish Conference and Annual Meeting of the Early Life History Section, American Fisheries Society, will be held in conjunction with the Annual Meeting of the American Society of Ichthyologists and Herpetologists.

The session's purpose is to present original research (and/or timely reviews) concerned with the anatomical, physiological, and neurobiological–neuroethological development of sensory systems in fish, and the coincident acquisition of behaviors based upon them. Studies that address the interaction between sensory system *and* behavioral ontogeny would be of particular interest, although papers concerned with any single subdiscipline will also be considered.

Dates: 24–28 June 1988; Place: Central Campus, University of Michigan, Ann Arbor, MI; *abstract deadline*: 1 April 1988.

Please direct abstracts or inquiries to: Howard I. Browman, Department of Systematics and Ecology, The University of Kansas, Lawrence, KS 66045, USA; telephone: (913) 864-4375.

1988 W. F. Thompson Award
American Institute of Fisheries Research Biologists

Best Student Paper Award: Call for Nominations

The W. F. Thompson Award Committee of the American Institute of Fishery Research Biologists (AIFRB) is soliciting nominations for the 1988 W. F. Thompson Award. Nominations must be received by 1 July 1988. The winner will be announced by 31 August 1988.

The award is for the best paper *published* by a student on any topic in the field of freshwater or marine fisheries science. The paper must have been published *within the last 3 years (1985–87)*, and must concern work done while the principal author was a student. Eligibility is not restricted to American citizens. Anyone may nominate qualified papers for consideration.

The winning author will receive a certificate from AIFRB and a check for $750.00.

Papers are evaluated on the basis of originality, organization, and development, and pertinence to current problems in fisheries.

Papers may have multiple authors, but the student nominated for the award must be the principal author. Where the winning paper is multiauthored and includes the academic advisor, the advisor will also receive a certificate acknowledging the advisor's role in producing the outstanding paper.

Please send five (5) copies of each nominated paper to Dr. Elizabeth F. Vetter, Chair, AIFRB W. F. Thompson Award Committee, Southwest Fisheries Center, P.O. Box 271, La Jolla, CA 92038, USA; telephone: (619) 546-7099.

Développement fonctionnel de systèmes sensoriels et l'acquisition de comportements chez les larves de poisson

Avis et demande de soumission de communications

Une séance spéciale de la 12e Conférence annuelle sur les larves de poissons et de la réunion annuelle de la Early Life History Section de l'American Fisheries Society aura lieu en même temps que la réunion annuelle de la l'American Society of Icthyologists and Herpetologists.

Cette séance spéciale servira d'enceinte à la présentation de recherches originales (ou de critiques opportunes) sur le développement anatomique, physiologique et neurobiologique–neuroéthologique de systèmes sensoriels chez les poissons et l'acquisition coïncidente de comportements basés sur ces systèmes. Les études portant sur l'interaction entre les systèmes sensoriels et l'ontogénie du comportement sont particulièrement sollicitées quoique celles touchant une sous-discipline particulière seront aussi examinées.

Date: du 24 au 28 juin 1988; endroit: Campus Central, Université du Michigan, Ann Arbor, MI; date limite de soumission des communications : 1er avril 1988.

Veuillez faire parvenir vos communications et vos demandes d'information à : Howard I. Browman, Department of Systematics and Ecology, The University of Kansas, Lawrence, KS 66045, USA; téléphone : (913) 864-4375.

Prix W. F. Thompson de 1988
American Institute of Fisheries Research Biologists

Prix pour la meilleure étude publiée d'un étudiant : appel de soumission

Le Comité du prix W. F. Thompson de l'American Institute of Fishery Research Biologists (AIFRB) reçoit actuellement les nominations pour le prix W. F. Thompson qui sera décerné en 1988. Les nominations devront être reçues d'ici le 1er juillet 1988. Le nom de la personne gagnante sera annoncée au plus tard le 31 août 1988.

Le prix vise à récompenser l'étudiant(e) ayant rédigé la meilleure étude *publiée* sur un thème lié aux pêches en eau douce ou marine; cette étude doit avoir été publiée au cours des *trois dernières années (1985–87)*. De plus, elle doit porter sur des travaux effectués pendant que le premier auteur était étudiant. La mise en nomination n'est pas restreinte aux citoyens américains et peut être faite par n'importe qui.

Le lauréat recevra un certificat de l'AIFRB ainsi qu'un chèque de 750 $.

Les communications sont évaluées en fonction de leur originalité, leur plan, leur contenu et leur pertinence aux problèmes courants des pêches.

Une communication peut avoir plusieurs auteurs mais l'étudiant(e) proposé(e) pour le prix doit en être l'auteur principal. Lorsqu'un(e) étudiant(e) reçoit un prix et un certificat pour une communication à plusieurs auteurs dont son conseiller académique, celui-ci recevra un certificat reconnaissant sa collaboration à l'étude primée.

International Symposium on Fish Physiology, Toxicology and Management

An International Symposium on Fish Physiology, Toxicology and Management will be held at Zhongshan University, Guangzhou, People's Republic of China, September 14–16, 1988. The language of the symposium is English. Invited speakers from six countries will present papers in symposium sessions on (1) Reproduction and Growth of Fish, (2) Physiology and Behavior of Fish, and (3) Toxicology and Risk Assessment in Aquatic Systems.

For further information, please contact: Dr. David Randall, Zoology Department, University of British Columbia, 6270 University Boulevard, Vancouver, B.C. V6T 2A9, Canada, or Dr. Lin Hoa-Ren, Biology Department, Zhongshan University, Guangzhou, People's Republic of China.

International Conference
Trace Metals in Lakes

August 15–18, 1988
National Water Research Institute
Burlington, Ontario, Canada

This is believed to be the first international conference devoted exclusively to the understanding of lakes as critical receptors of metal pollution. Contributions are invited on any aspects of the sources, fate, effects, and cycling of trace metals and metalloids in lake ecosystems. The main focus, however, will be on three themes: (1) our current understanding of the extent of trace metal contamination of lake ecosystems, and the methodological difficulties in trace metal determinations in freshwaters; (2) effects of lake acidification on the cycling and bioavailability of trace metals in lakes; and (3) similarities and differences in the linkages of trace metal and nutrient cycles in marine and lake ecosystems.

The Technical Program will consist of oral and poster presentations. The latter will be given equal prominence, and time will be allotted for display, review, and brief (about five minutes) discussion of these papers. Papers presented at the conference (including poster papers) may be submitted for *critical review* and subsequent publication as a special issue of the journal, *Science of the Total Environment*.

The registration fee will be Can$100.00 if paid by May 31, 1988, and Can$125.00 if payment is made at the conference. The last date for submission of abstracts is March 31, 1988. For guidelines on *preparation of abstracts* and for further information, contact Dr. Ken R. Lum, National Water Research Institute, P.O. Box 5050, Burlington, Ont. L7R 4A6, Canada; telephone: (416) 336-4617.

Cinq (5) exemplaires de la communication doivent être envoyés au Dr Elizabeth F. Vetter, présidente, Comité du prix W. F. Thompson de l'AIFRB, Southwest Fisheries Center, P.O. Box 271, La Jolla, CA 92038, USA; téléphone : (619) 546-7099.

Symposium international sur le poisson : physiologie, toxicologie et gestion

Un Symposium international sur le poisson : physiologie, toxicologie et gestion aura lieu du 14 au 16 septembre 1988 à l'Université Zhongshan, Guangzhou, République populaire de Chine. La langue de communication utilisée sera l'anglais. Les séances de communications présentées par les conférenciers invités de six pays porteront sur les sujets suivants : (1) la reproduction et la croissance des poissons, (2) la physiologie et le comportement des poissons et (3) la toxicologie et l'évaluation des risques dans des systèmes aquatiques.

Pour de plus amples informations, veuillez vous adresser aux personnes suivantes : Dr David Randall, Zoology Department, University of British Columbia, 6270, University Boulevard, Vancouver (B.C.) V6T 2A9, Canada, ou Dr Lin Hao-Ren, Biology Department, Zhongshan University, Guangzhou, People's Republic of China.

Conférence internationale
Métaux à l'état de traces dans les lacs

15 au 18 août 1988
l'Institut national de recherche sur les eaux
Burlington (Ontario), Canada

Il s'agira probablement de la première conférence internationale consacrée exclusivement à l'étude des lacs en tant que victimes de la pollution par les métaux. Des communications pourront être présentées sur différentes questions liées à l'origine, à l'évolution, à l'incidence, à la biotransformation et au mouvement des métaux à l'état de traces et métalloides dans les écosystèmes des lacs. La conférence portera principalement sur les trois grands thèmes suivants : (1) nos connaissances actuelles concernant l'étendue de la contamination des écosystèmes de lacs par les métaux à l'état de traces, ainsi que les difficultés que pose la mesure des métaux à l'état de traces en eau douce; (2) l'incidence de l'acidification des lacs sur la biotransformation, le mouvement et la biodisponibilité des métaux à l'état de traces dans les lacs; et (3) les similitudes et les différences dans les relations qui s'établissent entre les métaux à l'état de traces et les cycles des matières nutritives dans les écosystèmes marins et lacustres.

Le programme technique comprendra des exposés oraux et des séances de démonstration. On accordera à ce dernier groupe une importance égale au premier et du temps sera prévu pour l'affichage, l'étude et une brève discussion (environ cinq minutes) des sujets traités. Les communications présentées dans le cadre de la conférence (y compris les affiches) pourront être soumises à une *analyse critique* en vue de leur publication dans un numéro spécial de la revue *Science of the Total Environment*.

Le coût de l'inscription est de 100 $ Can avant le 1 mai 1988 et de 125 $ Can au moment de la conférence. La date limite pour la présentation des résumés est le 31 mars 1988. Pour recevoir des directives pour la présentation des résumés et pour d'autres renseignements, veuillez contacter Monsieur Ken R. Lum, Ph.D., Institut national de recherche sur les eaux, C.P. 5050, Burlington (Ont.) Canada L7R 4A6; téléphone : (416) 336-4617.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Jakobsen, P. J., G. H. Johnsen, and P. Larsson. Effects of predation risk and parasitism on the feeding ecology, habitat use, and abundance of lacustrine threespine stickleback (*Gasterosteus aculeatus*)

Hay, D. E., J. R. Brett, E. Bilinski, D. T. Smith, E. M. Donaldson, G. A. Hunter, and A. V. Solmie. Experimental impoundments of prespawning Pacific herring (*Clupea harengus pallasi*): effects of feeding and density on maturation, growth, and proximate analysis

Hay, D. E., and J. R. Brett. Maturation and fecundity of Pacific herring (*Clupea harengus pallasi*): an experimental study with comparisons to natural populations

Cornett, R. J., and L. Chant. ^{239,240}Pu residence times in freshwaters and accumulation in shield lake sediments

Harding, G. C., and R. W. Trites. Dispersal of *Homarus americanus* larvae in the Gulf of Maine from Browns Bank

Beyer, J. M., G. Lucchetti, and G. Gray. Digestive tract evacuation in northern squawfish (*Ptychocheilus oregonensis*)

Mulligan, T. J., S. McKinnell, and C. C. Wood. Uncertainty in stock composition estimates of oceanic steelhead trout using electrophoretic characteristics: comments on a recent study

Nürnberg, G. K. Prediction of phosphorus release rates from total and reductant-soluble phosphorus in anoxic lake sediments

Doyon, J.-F., J. A. Downing, and E. Magnin. Variation in the condition of northern pike, *Esox lucius*

Cunjak, R. A. Physiological consequences of overwintering in streams: the cost of acclimitization?

Steedman, R. J. Modification and assessment of an index of biotic integrity to quantify stream quality in southern Ontario

Dempson, J. B., E. Verspoor, and J. Hammar. Intrapopulation variation of the Esterase-2 polymorphism in the serum of anadromous Arctic charr, *Salvelinus alpinus*, from a northern Labrador river

Walters, C. J., J. S. Collie, and T. Webb. Experimental designs for estimating transient responses to management disturbances

Earle, J. C., H. C. Duthie, W. A. Glooschenko, and P. B. Hamilton. Factors affecting the spatial distribution of diatoms on the surface sediments of three Precambrian Shield lakes

Winters, G. H., and E. L. Dalley. Meristic composition of sand lance (*Ammodytes* spp.) in Newfoundland waters with a review of species designations in the Northwest Atlantic

Holtby, L. B. Effects of logging on stream temperatures in Carnation Creek, British Columbia, and associated impacts on the coho salmon (*Oncorhynchus kisutch*)

Prager, M. H., and A. D. MacCall. Sensitivities and variances of virtual population analysis as applied to the mackerel, *Scomber japonicus*

Stobo, W. T., J. D. Neilson, and P. G. Simpson. Movements of Atlantic halibut (*Hippoglossus hippoglossus*) in the Canadian North Atlantic

Notes

Armstrong, M. J., and P. A. Shelton. Bias in estimation of stock–recruit function parameters caused by nonrandom environmental variability

Smith, B. D. Comparison of productivity estimates for *Laminaria* in Nova Scotia

Trimbee, A. M., and E. E. Prepas. Dependence of lake oxygen depletion rates on maximum oxygen storage in a partially meromictic lake in Alberta

Welch, H. E., M. A. Bergmann, J. K. Jorgenson, and W. Burton. A Subice Suction Corer for sampling epontic ice algae

Montevecchi, W. A., D. K. Cairns, and V. L. Birt. Migration of postsmolt Atlantic salmon, *Salmo salar*, off northeastern Newfoundland, as inferred by tag recoveries in a seabird colony

Perspectives

Northcote, T. G. Fish in the structure and function of freshwater ecosystems: a "top-down" view **361–379**

Letters and comments/Lettres et commentaires

Webb, K. L. Comment on "Nutrient limitation of phytoplankton growth in brackish coastal ponds" by Caraco, Tamse, Boutros, and Valiela (1987) **380–381**
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